GRIGORE T. POPA UNIVERSITY OF MEDICINE AND PHARMACY IASI

# Habilitation thesis

# Multidisciplinary approaches in understanding the mechanistic of psychiatric disorders

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# List of Abreviations

4-HNE - hydroxynonenal
AD - Alzheimer's disease
ADAS-cog - Alzheimer's Disease Assessment Scale-Cognitive subscale
AIMS-abnormal movement scale
ATP - adenosine 5'-triphosphate
AUDIT - Alcohol Use Disorders Identification Test
BDNF - brain derived neurotrophic factor
BM – biometal
BPRS – brief psychiatric rating scale
COMT - catechol-O-methyl transferase
FST - Forced Swim test
GCP - good clinical practice
GPX- glutathione peroxidase
HPA - hypothalamic-pituitary-adrenal
HO− hydroxyl radical
H2O2 - hydrogen peroxide
MAO - monoamine oxidase A
MCI - mild cognitive impairment
MDA malondialdehyde
NAC - N-acetylcysteine
O2 − superoxide anion
OT - oxytocin
PANSS - Positive and negative syndrome scale
PUFA – polyunsaturated fatty acid
PD –Parkinson Disease
QOL -quality of life scales
ROCH - reactive aldehyde
ROO − peroxyl
ROS -reactive oxygen species
SOD superoxide dismutase
SSRI - serotonin reuptake inhibitor
ST - The splash test
TBARS - thiobarbituric acid reactive substances
TCT - three-chamber sociability test
TNFa - tumor necrosis factor-alpha
TRX -thioredoxin

# SECTION I: PROFESSIONAL, SCIENTIFIC AND ACADEMIC ACHIEVEMENTS

## Professional achievements

I graduated from the Faculty of General Medicine of the University of Medicine and Pharmacy "Grigore T. Popa" Iasi in 2000, and in the fall of the same year I was accepted for a doctorate under the guidance of Professor Dr. Vasile Chiriţă, with the research topic "Psychopathological, therapeutic and ethico-medical correlations in the recovery of the schizophrenic patient", a project in which I presented a series of scientific reports.

The doctoral thesis addresses the difficult problem related to the treatment of schizophrenia, an issue of great importance in the field of mental health and curative psychiatric care. Current trends in psychopharmacology consider atypical antipsychotics as first-line antipsychotics in the treatment of the first psychotic episode, because they are associated with less significant side effects, which may be the basis of therapeutic compliance and thus improved prognosis.

The current paper, involving the efficiency of atypical antipsychotics in schizophrenia is an integrated part of the abovemetioned field. This is a topic of fundamental importance, given that the use of classical neuroleptics in the clinical context is associated with side effects as a result of reduced selectivity of this class of drugs. Thus, the current trend is to use atypical antipsychotics increasingly frequently for their much fewer side effects and high selectivity.

In recent years, significant changes have been imposed in the conceptualization of schizophrenia care regarding communitary dimensions such as the reduction of hospitalization, outpatient treatment and socio-familial integration through "sheltered housing". The new ways of psychosocial assistance have been integrated in the idea of solving the social and psychological difficulties common to schizophrenic patients, so that this approach aims to limit the number of relapses and socio-familial and professional reintegration. Cases of long-term schizophrenia can be assisted in psychiatric hospitals or outpatient clinics and in community-based forms of care. These last forms of care, materialized in the economically developed countries, emphasize the extra-mural care and the limitation of the hospitalization for the benefit of the patients, the family and the society. Thus, the increased degree of independence and the opportunity for community integration effectively contribute to the process of psychosocial rehabilitation.

During 2001-2003 period I attended the master's courses "Psychosocial Intervention and Psychotherapy" within the Faculty of Psychology and Educational Sciences, University "Al. I. Cuza" from Iaşi in order to complete my academic studies and to perfect my knowledge and skills in the field.

This training in psychotherapy allowed me to obtain new knowledge that I was able to put into practice both for patients and in teaching students. Psychotherapy plays an important role in the treatment of schizophrenia in combination with antipsychotic medication. The obstacles to this therapy are the patient's distrust of the therapist and the patient's emotional distance from the therapist. On the other hand, it is considered that the possibility of establishing a therapeutic alliance is one of the factors of a positive prognosis. Nonetheless establishing a

patient-therapist relationship is a difficult process compared to the therapy of non-psychotic patients, the reasons being due to the patient:

- Solitary in nature;
- Suspiciousness;
- May be anxious or hostile;
- Closed in.

Sometimes the failure of psychotherapy has its roots in the inappropriate behavior of the therapist. The desire for premature information, inopportune addressing, exaggeratedly friendly and emotionally warm attitude can be interpreted deliriously. The therapist must arm himself with patience, sincerity, a flexible attitude, elements that are essential in establishing the therapeutic alliance. Some simple steps such as remembering the patient's date of birth, accepting, and offering small gifts, walking or simply being around the patient can increase their confidence in the doctor. The patient gradually gets used to the idea that the doctor wants to help him, to understand him, regardless of the bizarre or even hostile behavior that may occur at a certain time.

In 2006, after psychiatric residency training I passed the final exam, I became a "Psychiatrist" MD, confirmed by the Order of the Ministry of Health no. 1760/2006.

In 2011, I took and passed the primary doctor exam and I was confirmed by the Order of the Ministry of Health no. 1296/1 September 2011 a primary psychiatrist.

The continuous training also involved obtaining the certificate of complementary studies in "Palliative Care" in May 2012, after completing the 2 years of training at the Center "Hospice - House of Hope" in Brasov, under the supervision of coordinator Prof. Dr. Daniela Moşoiu. Palliative care aims to improve the quality of life of patients and their families by using a holistic approach: physical, mental, social and spiritual, within a multidisciplinary team.

In 2013, I took and passed the specialist doctor's exam in "Forensic Medicine" confirmed by the Order of the Ministry of Health no. 1/2014.

It was a challenge to complete a second medical specialty, but I considered that this is of great importance for a member of the psychiatric forensic expertise commissions. Psychiatric forensic expertise is the interdisciplinary activity of assistance and scientific research in which the responsibility and ethical conscience of experts is engaged in the highest degree. The ethics of psychiatric diagnosis is a matter of deontology of the expert and is part of the broader framework of deontology and medical responsibility.

The expert's responsibility does not differ from the responsibility of any citizen. The fact that the expert carries out a judicial mission does not confer any immunity on him. The quality of "auxiliary of justice" does not exonerate the expert from the mistakes he can make when exercising his functions, this being obvious in the strict framework of his missions. The progress made in the direction of investigating and treating the mentally ill, the topicality of international verification of the criteria and completion of psychiatric forensic expertise with implications in criminal law in the European Union's member countries also raises the issue of improving Romanian legislation regarding

1.the statute of the mentally ill (Reviewed Mental Health Law)

2. medical-social-legal assistance

3. institutional insurance framework (malpraxis insurance care which are signed more or less formally and do not cover the majority of risks the doctor is exposed to (a malpraxis insurance

policy designed specifically for members of the psychiatric forensic examination commission should exist, even if the insurance premium would be higher in cost).

The continuous training also involved obtaining the certificate of complementary studies in "Management of Health Services" in November 2017, after completing the 12 training modules within the National School of Public Health, Management and Training in Health Bucharest.

**Academic activity**

In 2003 I became a university assistant, through a competition, at the Psychiatry Discipline - Faculty of General Medicine within the University of Medicine and Pharmacy "Grigore T. Popa". The didactic activity consisted in guiding the groups of students from the Faculty of General Medicine and from the College of Nurses, the training consisting in practical activities and psychiatry seminars. From the very beginning, in collaboration with other colleagues in the discipline and under the guidance of Professor Dr. Vasile Chiriţă, we implemented new methods of approaching the curriculum, with necessary changes in the analytical curriculum for general medicine students, in accordance with modern classifications guides in psychiatry. At the same time, I actively participated in rethinking the methodology for examining the knowledge gained by students, in practical and theoretical terms, by developing multiple choice tests that include a broad coverage of the information needed by medical graduates.

In 2007 I took the competition of assistant professor at the Psychiatry Discipline - Faculty of Medicine. As a university assistant, I participated in teaching activities included in the curriculum, by conducting clinical internships of sixth-year students of the Faculty of Medicine and fourth-year students of the Colleges of Nurses. I guided the diploma papers of the students who were assessed at the undergraduate exam with a maximum grade. The diploma papers were focused on the research topic of the psychiatric clinic - current issues of nosology, etiopathogeny psychopathology and clinical care of mental illness, problems of psychopharmacology and psychotherapy.

During this period, I conducted a series of courses in the country and abroad, being trained in communication techniques, the presentation of medical materials both from the perspective of science, research, good clinical practice (GCP) and from the perspective of teaching. These aspects of professional training have given me the possibility of a modern approach to the curriculum of the discipline, applied to medical students who have completed the psychiatric internship.

In 2013 I took the competition for lecturer at the Psychiatry Discipline within the Faculty of General Medicine. The didactic activity as head of works diversified by giving classes with the students of the Faculty of Medicine, but at the same time I became a guide not only for the resident doctors in the psychiatric specialty, but also for the residents in other medical specialties.

Since 2013, I have been involved in the development and introduction of a new course direction, entitled "Palliative Care", a compulsory subject in the Faculty of Medicine, which I coordinated for 2 years, currently holding seminars and practical activity regarding this discipline of study. Currently the activity within the Palliative Care discipline takes place at the Regional Institute of Oncology Iași and the “Socola” Institute of Psychiatry Iași.

I took the optional course "Addiction Medicine" for third year students of the Faculty of General Medicine. I am adviser for the students of the 6th year, Faculty of Medicine. I participated in the works of the Congress of Medical Students (CONGRESSIS), being involved in the judging procedure of the works, together with my fellow psychologist from the SCOP department of the University of Medicine and Pharmacy "Grigore T. Popa" Iasi.

Regarding the writing activity, I published as editor in "Psychiatry - The essential in clinical presentations", publishing house of "Gr. T. Popa ", U.M.F. Iasi, 2018.

Also, I participated as a co-author in the creation of the following monographs and treatises:

- "Pain management in palliative care patients - for family doctors", „Gr. T. Popa", U.M.F. Iasi, 2017,
- "The moral and professional profile of the expert from the psychiatric forensic expertise committees in Romania", Gr. T. Popa ", U.M.F. Iasi, 2015,
- "Traditions and orientations in modern geriatrics", author Rodica Ghiuru, „Gr. T. Popa", U.M.F. Iasi, 2014,
- "The Essential in Psychiatry", author Cristinel Ștefănescu, „Gr. T. Popa", U.M.F. Iași, 2013,
- "Aggression and mental illness", authors Călin Scripcariu, Roxana Chiriţă, Vasile Chiriţă, Sedcom Libris Publishing House, 2012.
- "News in geriatrics", subchapter "Depression in the elderly", author Ioana Dana Alexa, „Gr. T. Popa", U.M.F. Iasi, 2011.
- "Treaty on Psychiatry", edited by Prof. Dr. Vasile Chiriţă and Prof. Dr. Roxana Chiriţă., Andrei Saguna Foundation Publishing House, Constanta, 2009.

In 2019 I become a University Lecturer in the Psychiatry discipline and in the teaching activity I made competent efforts to acquire by students the discipline program and specialized knowledge necessary for complex medical training, by teaching psychiatric semiology and clinical psychopathology, examination and investigation of the mentally ill patients, presentation of clinical cases, application of bio-, chemo- and psychotherapeutic treatment methods, approach of the doctor-patient relationship and knowledge of the psychological implications caused by somatic diseases.

**Scientific activities**

In 2007, following the defense of the doctoral thesis from April 28, 2007, I received the title of Doctor of Medicine, confirmed by MEC Order no. 1418 / 29.06.2007.

The scientific activity has been outlined since the beginning of the professional training, by participating in clinical research trials, starting with 2002, holding the positions of under investigator, rater, coordinator of clinical studies and principal investigator since 2013.

Thus, the research activity in the field of psychiatry starts practically with the beginning of the doctorate studies and is outlined through the participation with scientific papers at congresses, symposia, national and international conferences.

In 2010, during the participation in the 23rd Congress of the European College of Neuropsychopharmacology in Amsterdam, 28 Aug.- 01. Sep. 2010, I presented the paper "Oxidative stress status in schizophrenic patients treated with typical versus atypical

antipsychotics" which was published in European Neuropsychopharmacology, Volume 20, Supplement 3, August 2010, Pages S491-S492.

In 2010, during the participation 17th International Congress of Neuropathology (ICN 2010) Salzburg, Austria, 10th-15th September, I presented "Changes of some oxidative stress markers in the serum of patients with mild cognitive impairment and Alzheimer's disease" which was published in Brain Pathology, volume 20, page21, September 2010.

In 2011, I participated at 15th Congress of the European-Federation-of-Neurological-Societies (EFNS), Budapest, Hungary, between 10th-13th September, I presented the paper "Changes of some oxidative stress markers in patients with anxiety disorder" which was published in European Journal of Neurology, volume 18, page 425, supplement 2, September 2011.

In 2012, at 16th Congress of the European-Federation-of-Neurological-Societies (EFNS), Stockholm, Sweden, SEP 08-11, I published "Oxidative stress in patients with mild cognitive impairment and depression: a possible factor for increasing the risk of developing dementia?" in European Journal of Neurology, volume 19, page 473, supplement 1, September 2012.

In 2014 I participated in the 15th World Congress on Pain, with the work "Undiagnosed major depressive disorder could be frequent in older patients with unexplained pain complains", a work awarded by the organizers with 1000$.

In 2016, during the participation in the 29th Congress of the European College of Neuropsychopharmacology in Vienna, 17-20. September 2016, I presented the paper "Intraperitoneal oxytocin administration for 9 consecutive days is reducing memory deficits and anxiety in a methionine rat model of schizophrenia" which was published in European Neuropsychopharmacology, Volume 26, Supplement 2, October 2016, Pages S289-S290.

In 2017 I participated at 3rd Congress of the European-Academy-of-Neurology, Amsterdam, Netherlands, June, with the paper "Intranasal oxytocin administration reduces memory, anxiety and depression-related deficits in a valproic acid - induced perinatal model of autism" which was published in European Journal of Neurology, volume 24, page 371, supplement 1, July 2017.

The scientific activity, permanently carried out, is linked and associated with medical and didactic activity and materialized in obtaining the title of Doctor of Medicine (2007), as well as in the elaboration of studies and scientific papers as author and co-author, published in extenso in specialized journals indexed by ISI or BDI or communicated and published in extenso or abstract in the volumes of national and international scientific events.

The ability to guide students or young researchers is argued by the membership in the guidance committee of the research activity of some doctoral students. Regarding this specific activity, we contributed in the process of choosing and adapting the best study methods of their research topics.

Moreover, I participated, as a team member in several clinical studies, including:

- Erasmus + project, contract no. 2017-1-RO01-KA203-037382 entitled "Translating International Recommendations into Undergraduate Palliative Care Curriculum (EDUPALL)", takes place between 01.09.2017-31.08.2020, coordinator Transilvania University of Brasov, UMF partner "Grigore T. Popa" from Iași.

- IASP project, contract no. 16410/2016 with the title “Physicians Education for Pain in NE Romania (PEPNER)”, carried out in the period 2016-2017, UMF contractor “Grigore T. Popa” from Iaşi, project director Associated Professor. M.D. Vladimir Poroch.
- Grant PN-II-RU-TE-2014-4-1886 / 01/10/2015 "Complex study on the relevance of oxytocin administration in some animal models of neuropsychiatric diseases", project director Alin Ciobica, UAIC Iaşi.
- UMF internal grant, contract no. 30888/2014 entitled “Ethical management system model in the NE region health care institutions - a support for the improvement of the quality of service for patients and of CONAS accreditation references”, carried out between 01.01.2015- 30.12.2016, director of project Associated Professor. M.D. Vladimir Poroch,
- UMF internal grant, contract no. 29239/2013 entitled “Study of the moral and professional profile of the expert from the psychiatric forensic expertise commissions in Romania”, carried out between 01.01.2014- 30.06.2015, project director Lecturer M.D. Anamaria Ciubară.
- CNCSIS project, contract no. 61GR / 2006 entitled “Identifying risk factors and intervention strategies in social and educational interference of computer use in children and adolescents”, carried out in 2007-2008, UMF contractor “Grigore T. Popa” from Iaşi, project director Prof. M.D. Vasile Chiriță.

In summary, I participated in the elaboration of 8 books, over 40 articles published in extenso, out of which 26 ISI indexed articles, 12 as main author and 16 BDI indexed articles.

I am the editor of the journal "Bulletin of Integrative Psychiatry", indexed by BDI but also peer-reviewed by the Journal of Medical-Surgical and Romanian Biotechnological Letters.

The scientific activity was also appreciated through citations in specialized journals - 244 citations Web of Science Core Collection, through the H-index 7 Web of Science Core Collection.

The fields of scientific interest focused largely on the topic of the doctoral thesis but also on the quality of life of the patient with mental disorders, the particularities of the pathology specific to older patients and the ethical implications in psychiatric care.

I have collaborated at the university community level with colleagues from other medical specialties, especially in the clinical fields. This collaboration is not limited to the clinical activity but is also found in the elaboration of studies and scientific papers materialized in publications and communications in the country and abroad.

I am part of the doctoral guidance commissions together with the doctoral supervisors Prof. Dr. Roxana Chiriță, Prof. Dr. Cristinel Ștefănescu, Prof. Dr. Cipriana Ștefănescu, Prof. Dr. Beatrice Ioan. I was a scientific referent in the commission for public defense of the doctoral thesis coordinated by Prof. Dr. Cristinel Ștefănescu.

I am also involved in the organizational activity of „Grigore T. Popa” University of Medicine and Pharmacy by participating every year in the admission commissions for the for Romanian students as well as for the candidates of Romanian nationality from the Diaspora. Starting with 2020, I became a residency coordinator in the specialty of Psychiatry.

Regarding postgraduate activity I am involved in the organizational activity for the national residency examination as head of the local commission. I am frequently a lecturer in postgraduate courses organized by the Psychiatry Discipline, I lectured in the "Summer School in Psychiatry" - activity dedicated to resident doctors as well as young specialist doctors.

The training of resident doctors is completed by taking the speciality examination, for which I was nominated as a member of the examination commission by the UMF management. I was also part of the primary doctor examination commissions.

The preoccupation for the development of the Psychiatry Department also consisted in participating in examination committee for university assistants and lecturers. I contributed to increasing the visibility of the Discipline at national level by participating in the competition commissions for the position of associate professor at „Carol Davila" University of Medicine and Pharmacy, Craiova, University of Medicine and Pharmacy, Lucian Blaga University of Sibiu, and Transilvania University of Brasov.

Academic and professional achievements:

- Elected member in the Council the Medicine Faculty at „Grigore T Popa" UMF Iași between 2016 – 2020;
- Elected member in University Senate of „Grigore T Popa" UMF Iași (Decission no. 4/28.01.2020).

I am a member several of professional organizations:

- since 2003 – member of The Society of Physicians and Naturalists of Iaşi;
- since 2007 - member of Romanian Psychiatry Association
- since 2016 – member in Professional and Scientific Committee within the Order of Medical Doctors Iași.

My particular interest in psychiatric field is sustained by the fact the psychiatric diseases are disturbances that are still far from being fully understand and also they are serious diseases with great negative impact on individual functionality. Of course, there are being made steps in the direction of understanding the biological and molecular basis of the main psychiatric pathologies, but until now, there is no reliable biological marker than can be used in psychiatry and the diagnostic is being established based on clinical subjective criteria. Also, there are a few reliable therapies for some of the psychiatric diseases, but there are also cases, including dementia, schizophrenia or addiction where the treatment available may have serious limitation.

Regarding the way that we approach the management of the psychiatric patient, we must say that this is the most complex therapeutical process in all the medicine. The case management of the patient has in the center the patient and its unique psychological features that impose a special treatment plan that is adjusted according to the special need of the patient. It is of extreme importance to have all the relevant information regarding the patient and to integrate information obtained by a collection of evaluation instruments (interviews, questionnaire, scales, information obtained throw other sources).

When we are confronted with a new case, the therapeutical success is depending on fully understanding the patient, including the cultural background, social and economic status, stressors, family relations, childhood development, nutrition, academic history, medical background but also aspect such as personality, religion and spirituality, character, passions and hobbies, cognitive biases.

The process of knowing the patients is a team effort that engages not only psychiatrist but also nurses, psychologists, social worker, family and close friends of the patient but also may include teachers or work colleagues. Further one, a biological and clinical profile of the patients is made that have to be correlated to the other collected data. The integration of the obtained data is complex and made as a team effort, but ultimately the case management leader is the one that orientates the management recommendation that has to be adapted to the specific case and need of the patient.

A better understanding of the patient warrants a good management of the case and favors a better outcome. This is why we have to better characterize the patient especially concerning the biological status. Regarding this particular aspect, we do not have at the moment specific biomarkers for the diagnosis of a particular psychiatric disease nor other parameters that may orientate choosing a specific psychotropic drug for the patient. Also, we are interested in finding some biological marker for prognostic, evolution and also assess some feared complication is psychiatry such as aggression or suicide.

It is still very useful a general biological profile, to know the general functioning of the organs, but certainly not enough. Despite, a real progress regarding pharmacotherapy in psychiatry, there are many gaps regarding these specific markers and also, for certain psychiatric diseases and for certain patients the vast majority of the psychiatric treatment fails. That is why, it is so important to have a better understanding of our case and our research is trying to increase the level of knowledge concerning the aspects I have already mentioned and to orientate and improve management of the psychiatric patient.

Considering these aspects our focus in on better understanding underlying mechanisms in these psychiatric conditions including oxidative stress in the main psychiatric diseases and possible antioxidant therapies, bio metals, nanoparticles, oxytocin relevance, the relation between the most important neurotransmitters serotonin and dopamine in the suicidal and aggressive patients. Also, I explored some new technique of treatment in alcoholism and also some metabolic disturbances considering that in psychiatric patients this is a frequent problem that complicates the course of the psychiatric condition.

Moreover, I studied some animal models in affective disorders and cognitive impairment from a practical but also theoretical perspective that may represent a translational model for future research. In this way, we analyzed the cognitive and antioxidant effects effect of an extract from plants that may have some therapeutic properties in a model of amnesia in mice considering the cognitive protective effect that we observed.

The oxidative stress paradigm in psychiatry is a special issue that me and our collaborators have studied in the last years considering the promising results that we observed in several psychiatric condition but also the data provided by the scientific community. We know that in psychiatric pathology there are broad and multiple mechanisms that contribute to the clinical manifestation of a certain disease.

Despite of the diversity of the psychiatric pathology, many clinical syndromes overlap in certain points, and we speculate that there are common pathways that can explain several psychiatric disorders. We have been focusing mainly on oxidative stress in this matter and we investigated the importance of oxidative stress in condition such as alcoholism, affective disorder, cognitive impairment or schizophrenia.

The involvement of oxidative stress in several psychiatric disorder is sustained by the evidence of increased peroxidation markers and an imbalance in a prooxidant-antioxidant system with increasing prooxidant products and usually a decreased in the capacity of oxidant enzymes. Evidence of oxidative stress have been found both in animal models but also in human patients with confirmed diseases. Understanding how oxidative stress is involved in psychiatric pathology could be an important step for diagnostic purposes but also in the psychotropic therapeutic field.

Describing models of oxidative impairment in some diseases, such as alcoholism or cognitive impairment may also give clues on the evolution on the condition, and this is exactly what we did in the present scientific report.

# SECTION I: CHAPTER I
# Analyzing the importance of oxidative stress in psychiatry

## 1.1 Introduction

Oxidative stress has been recently in the spotlight in several medical fields, especially in neurology and psychiatry. The understanding of oxidative stress relevance in psychiatric disorders is mandatory, since a growing amount of evidence support the idea that oxidative stress could be an important event in the pathogenesis of many psychiatric disorders. Generally, it is believed that various tissues have different susceptibilities to oxidative stress and that the brain is particularly more vulnerable to oxidative damage due to relatively low levels of antioxidants, high levels of polyunsaturated fatty acids, high metal content and an increased level of oxygen usage (Smith 2006, Sultana et al. 2008, and Hritcu et al. 2009).

Oxidative process happens all the time in the body and in a healthy person there is a strict control of the process that imped the toxic end terminal products to produce lesions to the normal tissue. The oxidative stress is the condition arising from imbalance between toxic reactive oxygen species (ROS) and antioxidant defense system.

Of course, we must understand that oxidative stress is just one part of a bigger process and that many other changes in the biology of the brain happen in order to trigger the disease. Oxidative stress and other reactions are interconnected, but trying to limit one, may have an important impact on other process determining limited clinical consequences.

For example, it was previously and very recently showed that the various mechanisms of oxidative stress and its therapeutic targets can be involved in aspects ranging from intracerebral hemorrhage (Yao et al, 2021) to its correlations to neuroinflammation (in relation or not with dementia- Ganguly et al. 2021) and going even to aspects related to the new COVID-19 pandemics (though its correlations with NOX 2 – Sindona et al., 2021 or other related mechanisms – Cechini et al., 2020, Forcados et al., 2021).

But why we focus so much on oxidative stress, if there are many other factors contributing to disturbances associated with psychiatric diseases?

The answer lays in the fact that it could be easier to have access to exogenous antioxidant and if that prove to be efficient, we can have important clinical benefit. Also, it is somehow easy to determine the level of antioxidant capacity or the lipid peroxidation marker and this is proving to have consistent importance in some psychiatric disorders including schizophrenia, then a set of biomarkers could be easily assessed.

And it is important to mention that no single disease in psychiatry, especially schizophrenia is not diagnosed using any biological marker, that is because no biological measurement proved yet to have a valid and sustained relevance in any psychiatric disturbances. That is why it could be very valuable to have a better understanding of oxidative stress in diseases such as schizophrenia, as we will describe it in the first chapter of the present work.

## 1.2. Assessing of oxidative stress markers in schizophrenia

### A. Background

Particularly, in some psychiatric condition such as schizophrenia that has a huge burden on patient, family and society in general, a better understanding of the pathogenesis of this disease could improve the management of schizophrenic patients.

There are evidences that oxidative stress may be involved in the etiopathogeny of some psychiatric diseases, such as schizophrenia and a better understanding of the relation of oxidative stress with schizophrenia may bring important advantages in the treatment approach of this disease.

Schizophrenia is a common psychiatric disorder that starts early in life and has a huge impact on the normal function of the individual but also is an enormous burden for society. Schizophrenia is manifested as gross distortion from reality; disturbances in thinking, feeling and behavior, has a chronic course and frequently relapses.

It is believed that increased oxidative stress may be relevant to the pathophysiology of schizophrenia, but most of the results regarding this subject are contrasting (Kunz et al. 2008, Dadheech et al. 2008, Wood et al. 2009, Padurariu et al. 2010). Considering that schizophrenia is in fact a spectrum of disorders with several subtypes and different forms of manifestations and also different ways of evolution, it is understandable that oxidative stress status may differ among schizophrenic patients.

Some of the lipoperoxidation metabolites include malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), which are considered the most specific and sensitive measures of lipid autooxidation (Haliwel et al. 2007, Zarkovic 2003). The potential toxicity of free radicals is counteracted by a number of cytoprotective enzymes and antioxidants that limit the damage. This protective mechanism function cooperatively in the form of a cascade which includes various antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPX) (Dadheech et al. 2008, Ciobica et al. 2009)

Considering that schizophrenia is the neuropsychiatric disorder with the most contrasting evidences regarding oxidative stress implication, in comparison with other disorders like Alzheimer's disease (which is characterized by a general reduction of the enzymatic antioxidant defense system and an increase of lipid peroxidation processes) (Baldeiras et al. 2008, Greilberger et al. 2008) or Parkinson's disease (where free radicals are leading to oxidative damage of dopaminergic neurons in the substantia nigra, increased lipid peroxidation and a decrease in glutathione concentration) (Seet et al. 2010, Ciobica et al. 2011).

Thus, based on our previous experience regarding the implication of oxidative stress in other neuropsychiatric diseases like mild cognitive impairment, Alzheimer's disease (Padurariu et al. 2010a), Parkinson's disease (Hritcu et al. 2008), anxiety (Ciobica et al. 2010, 2011), sleep or depression disorders (Vitalaru et al. 2010), we tried to summarize the most important aspects and discrepancies, regarding some oxidative stress markers in schizophrenia, by referring mainly to antioxidant enzymes modifications and lipid peroxidation markers, but also to analyze a few aspects regarding animal models and possible antioxidant therapy.

## B. Published articles in this field

Our interest in this domain has been materialized in the publication of a study which intended to evaluate the markers of oxidative stress in schizophrenia by the means of a systematic literature review.

Ciobica A, Padurariu M, Dobrin I, Stefanescu C**, Dobrin R**. Oxidative stress in schizophrenia - focusing on the main markers. Psychiatria Danubina 2011 Sep;23(3):237-45, **IF 2011 = 0.444**

- **Introduction**

Oxidative stress is the condition arising from imbalance between toxic reactive oxygen species (ROS) and antioxidant systems. Various tissues have different susceptibilities to oxidative stress. Brain is particularly more vulnerable to oxidative damage due to relatively low levels of antioxidants, high levels of polyunsaturated fatty acids, high metal content and oxygen utilization (Smith 2006, Sultana et al. 2008, Hritcu et al. 2009).

- **Material and method**

Thus, in one of our first papers on this matter we analyzed the main scientific databases (e.g. Pubmed, Sciencedirect, Scopus, Google Scholar), until July 2011, by using the following keywords "oxidative stress schizophrenia", "superoxide dismutase schizophrenia", "glutathione peroxidase schizophrenia", "4-hydroxynonenal schizophrenia" and "malondialdehyde schizophrenia". Cross-references for these key words were also counted in.

- **Results**

We present below (Table 1.1) the results summary of some important studies regarding oxidative stress profile in some schizophrenia studies:

Table 1.1. The status of the main oxidative stress markers in some schizophrenia studies

| Study | Oxidative stress marker | Status (vs. control, unless otherwise stated) |
|---|---|---|
| **Dadheech et al., 2008** | SOD | decreased activity in the red cells of never-treated schizophrenic patients |
| | GPX | decreased activity in the red cells of never-treated schizophrenic patients |
| | MDA | increased concentration in the blood of never-treated schizophrenic patients |
| **Gama et al., 2006** | SOD | increased activity in the serum of schizophrenic patients in partial psychotic symptoms remission |
| | | no significant modifications in the serum as a result of haloperidol vs. clozapine treatment |
| | TBARS | increased concentration in the serum of schizophrenic patients in partial psychotic symptoms remission |
| | | increased levels in in the serum of patients treated with clozapine as compared to those treated with haloperidol |
| **Gama et al., 2008** | SOD | no significant modifications in the serum among different subtypes (paranoid, disorganized, undifferentiated) |
| | | no significant modifications in the serum between different clinical course pattern (partial remission, marked symptoms and deteriorated) |
| | TBARS | no significant modifications in the serum among different subtypes (paranoid, disorganized, undifferentiated) |
| | | increased at the serum level in the subgroup with marked symptoms vs. deteriorated group |
| **Kunz et al., 2008** | SOD | increased activity in the serum of treated schizophrenic patients |

| | | |
|---|---|---|
| | TBARS | increased activity in the serum of treated schizophrenic patients |
| **Martins et al., 2008** (in rats) | TBARS | increased concentration in the striatum after haloperidol treatment |
| | | decreased concentration in the prefrontal cortex after olanzapine and aripiprazole treatment |
| | | decreased levels at the cerebral cortex level after haloperidol, clozapine, olanzapine and aripiprazole administration |
| | | no significant modifications in the hippocampus levels as a result of haloperidol, clozapine, olanzapine and aripiprazole administration |
| **Medina-Hernandez et al., 2017** | MDA + 4-HNE | increased concentrations in the serum of schizophrenic patients |
| | | increased concentration in the serum of treatment refractory schizophrenics, as compared to non-refractory schizophrenics |
| **Miljevic et al., 2010** | SOD | increased activity in the plasma of schizophrenic patients chronically treated with clozapine |
| | GPX | decreased activity in the plasma of schizophrenic patients chronically treated with clozapine |
| | CAT | no significant modifications in the plasma of schizophrenic patients chronically treated with clozapine |
| **Padurariu et al., 2010** | SOD | increased activity in the serum of schizophrenic patients of paranoid subtype |
| | | increased activity in the serum of schizophrenic patients treated with haloperidol and quetiapine |
| | GPX | decreased activity in the serum of schizophrenic patients of paranoid subtype |
| | | decreased activity in the serum of schizophrenic patients treated with haloperidol and risperidone |
| | MDA | increased levels in the serum of schizophrenic patients of paranoid subtype |
| | | increased levels in the serum of schizophrenic patients treated with haloperidol, quetiapine, olanzapine and risperidone |
| **Pazvantoglu et al., 2009** | TAOP | no significant differences in the serum of schizophrenic patients free of treatment for at least 2 weeks |
| | TPEROX | no significant differences in the serum of schizophrenic patients free of treatment for at least 2 weeks |
| **Pavlovic et al., 2020** | SOD | increased activity in the red cells of schizophrenic patients with positive symptoms |
| | | no significant modifications in the red cells of schizophrenic patients with negative symptoms |
| | GPX | decreased activity in the red cells of schizophrenic patients with both positive and negative symptoms |
| | MDA | no significant changes in the erythrocytes of schizophrenic patients with both positive and negative symptoms |
| **Radonjic et al., 2010** | GPX | decreased at the hippocampus level(animal model – phencyclidine administration) |

| | | |
|---|---|---|
| | MDA | increased at the hippocampus and thalamus level |
| **Rafa et al., 2009** | SOD | decreased activity in the plasma of treated or untreated schizophrenic patients |
| | GPX | decreased activity in the plasma of treated schizophrenic patients |
| | | no significant differences vs. control in the plasma of untreated schizophrenic patients |
| **Singh et al., 2008** | SOD | decreased activity in the serum of schizophrenic patients chronically treated with haloperidol |
| | TBARS | increased concentration in the serum of schizophrenic patients chronically treated with haloperidol |
| **Wang et al., 2019** | 4-HNE | increased levels in anterior cingulated brain of schizophrenic patients |
| **Zhang et al., 2006** | SOD | decreased activity in the plasma of chronic schizophrenic patients |
| | GPX | decreased activity in the plasma of chronic schizophrenic patients |
| | MDA | increased concentration in the plasma of chronic schizophrenic patients |
| | SOD and GPX | decreased activity in the plasma of schizophrenic patients with paranoid and residual subtype vs. disorganized subtype |
| | SOD, GPX and MDA | no significant modifications at the plasma level regarding the differential effects of typical vs. atypical antipsychotics |
| **Zhang et al., 2009** | TRX | increased in the serum of never medicated first episode schizophrenic patients |
| | | no significant modifications in the serum of chronic medicated schizophrenic patients |
| SOD = superoxide dismutase, GPX= glutathione peroxidase, MDA= malondialdehyde, TBARS = thiobarbituric acid reactive substances, 4-HNE = 4-hydroxynonenal, CAT= catalase, TAOP = total antioxidant potentials, TPEROX = total peroxide levels, TRX = thioredoxin. | | |

- ## Discussions

### 1. Lipid peroxidation markers

The production of reactive species is a core part of mitochondrial energy generation, and these species are dealt with by the body in multiple ways. These include SOD, that catalyses the conversion of superoxide radicals to hydrogen peroxide, which is then converted into water by GPX and catalase. Production of reactive oxygen species and the enzymatic defense mechanism against oxidative stress damage (Haulica et al. 2000).

While there are strong links between oxidative stress anomalies and the pathophysiology of schizophrenia, in vivo measurement of free radical concentrations is impractical because their reactive nature results in short half-lives and low levels. Oxidative status in clinical populations are typically assessed in other ways, such as the measurement of oxidative defences, particularly key enzymes we mentioned above (e.g. SOD, GPX, catalase), completed by assessment of the consequences of oxidative stress such as plasma lipid peroxides (Wood et al. 2009).

Studies performed in patients with schizophrenia have generally suggested the presence of a compromised antioxidant system (Pavlovic et al. 2002, Gysin et al. 2007, Dadheech et al.

2008, Wood et al. 2009), but this is not always consistent with specific observed parameters, which on the whole, showed evidences of dysregulation. In this way, for SOD, the first line of defense against ROS, both decreased (Ranjekar et al. 2003, Zhang et al. 2010) and increased (Reddy et al. 1991, Zhang et al. 2003) specific activities were reported. Moreover, studies stating no changes in SOD activity of patients with schizophrenia are mentioned (Yao et al. 1998).

Similar contrasting aspects are also described for GPX (Herken et al. 2001, Gawryluk et al. 2011) or catalase, with results presenting reduced (Reddy et al. 1991) or increased (Herken et al. 2001) levels in patients with schizophrenia, compared to control groups. In this way, increased antioxidant activity may reflect a preceding cellular oxidative stress or serve as a compensatory mechanism (Kuloglu et al. 2002, Dakhale et al. 2004, Rukimi et al. 2004, Kunz et al. 2008). Probably, this difference in various results can be attributed to different clinical symptoms, therapeutic features or duration of the illness. In addition, in a recent paper published by Pazvantoglu et al. (2009), it was demonstrated that the severity of symptoms was associated with the decreased antioxidant level.

Various antioxidants have been found to be related to negative symptoms, poor premorbid functions and computed tomography abnormalities. However, no significant relationship between duration of the disease and antioxidant levels was found. Also, antioxidants were reported to be different between various subtypes of schizophrenia: SOD and GPX activities were significantly lower in paranoid and residual subtypes, compared to both disorganized subtype and the control groups (Pazvantoglu et al. 2009). Also, our group previously reported contrasting results regarding the antioxidant enzymes, with a significant increase of SOD specific activity and a decrease of GPX activity in patients with schizophrenia, compared to a control group (Padurariu et al. 2010b).

Thus, we were interested in our previous research to find the extent of the oxidative stress in patients diagnosed with schizophrenia. Estimating levels of reactive oxidative products provides a very useful strategy to determine the impact of oxidative stress in these patients. Lipid peroxidation is often assayed by measuring thiobarbituric acid reactive substances (TBARS). The end products of lipid peroxidation, such as MDA assessment, have been very widely used indices of oxidative stress in clinical studies (Padurariu et al. 2010a, Baldeiras et al. 2008, Greilberger et al. 2008). Also, one of the most important products of lipid oxidation is 4-HNE, which is a highly cytotoxic reactive α,β-aldehyde that is generated during various physiological and pathophysiological conditions based on the production of ROS (Schaur et al. 2003, Siems et al. 2003).

Besides its important implications in Alzheimer's disease (where it can damage the cholinergic neurons by membrane permeabilization and apoptosis or cause impaired glutamate and glucose transport) (NegreSalvayre et al. 2010) or Parkinson's disease (it is found in the Lewy bodies and mitochondria of PD patients) (Zarkovic 2003), 4-HNE seems to exert important actions also in schizophrenia, considering that increased levels of 4-HNE were found in patients with schizophrenia, as compared to normal (significantly increased by more then 47% vs. controls in the study of Wang et al. from 2009), while Medina-Hernández et al. (2007) demonstrated an increase of 4-HNE concentration in treatment-refractory schizophrenics, as compared to non-refractory schizophrenics or with healthy controls, and also a significant

correlation between the increased lipoperoxidation processes and delusions or emotional withdrawal symptoms (Medina-Hernández et al. 2007).

Additionally, in the study of Wang et al. the separated analysis of the non-treated patients with schizophrenia revealed also an increase of 4-HNE levels in these subjects, suggesting that the increased 4- HNE in schizophrenia is not a result of the treatment and could be extremely relevant in the schizophrenic pathology. Also, elevated levels of MDA have been shown in plasma, erythrocytes, leucocytes and platelets of patients with schizophrenia (Kunz et al. 2008). We also demonstrated similar results in the serum of schizophrenic patients (Padurariu et al. 2010b).

Moreover, it is believed that a high level of TBARS is a sign of peroxidative injury to membrane phospholipids. Neuronal functioning is affected by this injury either by changes in membrane fluidity or by alterations in membrane receptors (Mahadik et al. 2001), which can cause neurotransmitter uptake, release impairment and even cell death (Gama et al. 2006). Some authors demonstrated that the extent of lipid peroxidation is positively correlated with the severity of symptoms in never-medicated patients and inversely with the levels of membrane-essential polyunsaturated fatty acids (Wood et al. 2009).

However, few studies reported lipid peroxidation only in rats chronically treated with haloperidol, but not in animals treated with risperidone, olanzapine or clozapine (Parikh et al. 2003). As in the case of the antioxidant enzymes, these contrasting results may be due to differences in species used for each study (rats and humans), different tissues (brain and serum), therapeutic features or duration of the illness.

**2. Differences between untreated and treated patients with schizophrenia**

We are also interested in the differences between untreated and treated patients with schizophrenia or the differences exerted by typical vs. atypical treatment on oxidative stress status. Differences between untreated and treated schizophrenic patients As we mentioned earlier, some contrasting results were obtained regarding oxidative stress status in patients with schizophrenia.

One of the responsible factors for this inconsistency could be the difference between untreated and treated schizophrenic patients. Generally, most of the aforementioned studies were performed on treated patients. In a very interesting study published in 2009, Raffa and colleagues studied these oxidative stress markers in neuroleptic-free schizophrenic patients (n=36), compared to healthy (n=46), but also schizophrenic treated patients (n=52). They found that comparing to the healthy controls, the patients with schizophrenia showed significantly lower levels of SOD, catalase and reduced glutathione.

Among the schizophrenic patients, the activities of the SOD and catalase were recorded to be significantly lower in untreated patients than in the treated ones, suggesting that a decrease in the glutathione levels and the activities of the antioxidant enzymes in patients diagnosed with schizophrenia is not related to neuroleptic treatment and could be considered as a biological indicator of the degree of severity for the symptoms of schizophrenia (Raffa et al. 2009).

This contradicted the idea that oxidative stress in patients with schizophrenia could be exacerbated further by treating them with antipsychotics, which possess prooxidant properties, based mostly of some papers reporting some possible oxidative stress induced by typical

antipsychotics treatment in both humans (Kropp et al. 2005) and rats (Parikh et al. 2003, Pillai et al. 2007).

The Raffa group results were also confirmed by another 2 recent studies, one lead by Dadeech et al., which used patients with schizophrenia that had never taken any treatment and had come for consultation for the first time (they found increased levels of MDA and decreased activities for both SOD and GPX) (Dadeech et al. 2008) and one that determined the levels of a novel oxidative stress marker, thioredoxin (TRX) in never-medicated first-episode schizophrenic patients, led by Zhang and colleagues.

They found that serum TRX is significantly increased in first-episode drug naive schizophrenia patients and greater compared to chronic schizophrenic patients on antipsychotic medication. Moreover, TRX levels did not differ between chronic patients and controls (Zhang et al. 2009). These results suggest that the oxidative stress markers could be used to indicate the degree for the severity of the disease in untreated patients with schizophrenia.

Some other authors affirm that excessive free radical production or oxidative stress may not be directly associated with the presence of the schizophrenia, but with the subtypes and/or the severity of the disorder (Pazvantoglu et al. 2009). However, another study from Zhang et al. reported an increase in the SOD levels in neuroleptic-free schizophrenic patients (Zhang et al. 2003). This could be explained by the short neuroleptic-free period the authors used (two weeks-time in which the status of being drug-free might not have been long enough to allow the antioxidant enzymes to revert to their normal levels).

### 3. The differences in schizophrenic patients treated with typical vs. atypical antipsychotic treatment

Concerning the differences in schizophrenic patients treated with typical vs. atypical antipsychotic treatment on oxidative stress in addition to the differences observed in treated vs. untreated schizophrenics, there are also controversies regarding the oxidative stress status in patients treated with typical vs. atypical antipsychotics.

Some of the authors reported that chronic administration of typical antipsychotic haloperidol, but none of some new generation antipsychotic like risperidone, clozapine or olanzapine induces oxidative stress by decreasing the activity of antioxidant enzymes and cause membrane lipid peroxidation (Parikh et al. 2003). However, there are studies demonstrating a decreased level of lipid peroxidation in the cerebral cortex, as a result of haloperidol chronic administration (Martins et al. 2008), as well as significant reduction of GPX specific activity in the blood of long-term clozapine treated schizophrenic patients (Miljevic et al. 2010).

Others hypothesized that the increased oxidative stress observed in some clozapine-treated groups could be indicative of illness severity, since this drug is formally indicated for a specific group of patients with schizophrenia, whose symptoms are refractory to other neuroleptics (Gama et al. 2006).

However, in a very complex study by Zhang et al. in 2006, no significant differences in plasma MDA levels and SOD, GPX and catalase activities were found among almost 100 patients from three subgroups treated with clozapine, risperidone and typical antipsychotics. It seems that both typical and atypical antipsychotic drugs may, at least partially, normalize the abnormal free radical metabolism in schizophrenia without a significant difference in their effects. Taken together, it is likely that pharmacological mechanisms of typical and atypical

antipsychotics may be different, but end point effects on oxidative stress could be the same. Also, in one of our previous studies we reported no major differences between patients treated with typical vs. atypical antipsychotics (Padurariu et al. 2010b).

**4. Animal models**

For further research in this area, animal models of schizophrenia have been described. These could be very helpful in order to increase the accuracy of studies regarding the involvement of oxidative stress in different aspects of schizophrenia.

Phencyclidine administration to rodents represents one of the most suitable animal models of schizophrenia among other models described in the literature. Phencyclidine has psychotomimetic properties and is capable of producing both positive and negative symptoms of schizophrenia, exacerbating existing psychoses in schizophrenics or inducing cognitive dysfunctions in healthy volunteers.

In this way, Radonjić et al. reported alterations in SOD activity and in the levels of lipid peroxides in different central regions of rats, as a result of postnatal treatment with phencyclidine (Radonjić et al. 2010). Similar aspects regarding glutathione depletion in rats were also recently reported (Castagne et al. 2004, Cabungal et al. 2007, Dean et al. 2009).

In addition, animal models can be used for understanding the effects of antipsychotics long-term treatment on the expression of antioxidant enzymes and oxidative neural cell injury. This could be important for explaining the possible differential mechanisms underlying some clinical side effects, but also planning long term-use or switch over between antipsychotics in the management of schizophrenia (Pillai et al. 2007). Still, as schizophrenia is a complex disease, animal models can only serve as models to a certain extent.

**5. Antioxidant treatment**

Also, one of the most important aspects in investigating oxidative stress in psychiatric disorders such as schizophrenia represent the potential therapeutic effect of some antioxidants. In this way, some authors suggested that the use of antioxidants might provide an improvement in the treatment of schizophrenic patients.

For example, Zhang and colleagues demonstrated in two different studies from 2001 that adding a Ginkgo biloba extract (well known for its antioxidant effects-Mantle et al. 2003) to classical haloperidol treatment, results in enhancing the effectiveness of the antipsychotic and reduces some extrapyramidal side effects (Zhang et al. 2001a). Moreover, adding Ginkgo biloba extract also resulted in better scores in the Scales for the Assessment of Positive and Negative Symptoms and decreased SOD levels (Zhang et al. 2001b).

The use of essential polyunsaturated fatty acids has also been suggested, considering that dysregulation of membrane phospholipid metabolism exists throughout the body from the onset of psychosis in patients with schizophrenia (Mahadik et al. 2003).

Reduced levels of membrane essential polyunsaturated fatty acids like arachidonic acid, eicosapentaenoic acid, docosapentaenoic acid or docosahexaenoic acids and their association with psychopathology have been consistently reported in both chronic-medicated schizophrenic patients as well as in never-medicated patients soon after the first episode of psychosis (Arvindakshan et al. 2003).

These compounds are highly enriched in the brain and are crucial for brain and behavioral development (Reddy et al. 2010). A phospholipid metabolic defect may already exist before the start of psychosis, even at the embryonic stages (Mahadik et al. 2003). Because these membrane phospholipids play a crucial role in the membrane receptor-mediated signal transduction of several neuro-transmitters and growth factors, their altered metabolism may contribute to the reported abnormal information processing in schizophrenia (Peet et al. 2001, Emsley et al. 2002, Bitanihirwe et al. 2011).

In this way, some authors reported that the severity of symptoms seems to correlate with the membrane arachidonic acid and docosahexaenoic acid levels, which are influenced by patient's dietary intake and lifestyle (Joy et al. 2006, Freeman et al. 2006, Berger et al. 2007).

In addition, a combination of eicosapentaenoic/docosahexaenoic acid and vitamin C/E resulted in a significant reduction of schizophrenia psychopathology, suggesting that (Ciobica et al. 2011) essential polyunsaturated fatty acids supplementation could represent a very effective treatment to improve the outcome of the disease for an extended period of time (Arvindakshan et al. 2003).

In fact, there are ungoing clinical trials on the benefit of fatty acids on the negative symptoms in schizophrenia which may lead to the future management of schizophrenia, especially where there are more negative symptoms.

Also, the use of alfatocopherol alone is mentioned in some studies, especially for treating tardive dyskinesia that may appear as a side effect of antipsychotics long-term use (Yao et al. 2001). Additionally, Amminger and colleagues published in 2010 a double-blind, placebo-controlled trial conducted for over 4 years in young people with ultrahigh risk of psychotic disorder, in which they report a significant decrease in the rate of progression to psychotic disorder as a result of long-chain omega-3 fatty acids administration (Amminger et al. 2010).

The above findings provide further evidence for a role of free radical-mediated psychopathology in schizophrenia and its complications. However, despite being promising, these studies require independent.

In conclusion, oxidative stress seems to be a key component in the schizophrenia pathophysiology. It is generally believed that oxidative stress may constitute a central point where other factors of vulnerability meet and their interactions could play a decisive role in the schizophrenic pattern.

**C. Published paper in this field**

Upon further examination on the subject, we decided to look into the specifics of oxidative stress status in schizophrenia depending on the generation of the antipsychotic used for treatment.

M.Padurariu, I.Dobrin, C.Stefanescu, A.Ciobica, **R.P.Dobrin** - "Oxidative stress status in schizophrenic patients treated with typical versus atypical antipsychotics" which was published in European Neuropsychopharmacology, Volume 20, Supplement 3, August 2010, Pages S491-S492

- **Introduction**

Schizophrenia is a common psychiatric disorder, marked by gross distortion from reality; disturbances in thinking, feeling, and behavior. However, the chemical nature of the

schizophrenic brain is still not completely understood. Some authors suggested that the damage caused by the occurrence of oxidative stress might be the biochemical basis of neurodevelopmental abnormalities that are manifested in schizophrenia.

Also, it is believed that oxidative stress may contribute to specific aspects of schizophrenic symptomatology and complications of its treatment. However, there are very few studies that compared the effects of typical vs. atypical antipsychotics on peripheral oxidative stress markers. In the present study we wanted to investigate whether there are any differences in the effects of typical (haloperidol) vs. atypical (quetiapine and olanzapine) antipsychotics on antioxidant enzymes activities and lipid peroxidation process.

- **Material and Methods**

30 schizophrenic patients in partial psychotic symptoms remission were selected using DSM-IV criteria for schizophrenia. They were of the chronic type, with duration of illness for at least 5 years and age between 25−60 years. All patients had been receiving stable doses of oral neuroleptic medications for at least 12 months prior to entry into the study. 15 patients were under haloperidol (approx. 10 mg daily dose) treatment and 15 patients were under quetiapine (approx. 300 mg daily dose) or olanzapine (approx. 20 mg daily dose) treatment. We assessed the levels of some enzymatic antioxidant defences like superoxide dismutase (SOD) and glutathione peroxidase (GPX), as well as lipid oxidation makers like MDA (malondialdehyde), from the patients peripheral blood, using chemiluminometric and spectrophotometric methods. The results were compared to a normal control group, which was mainly recruited form the hospital staff, and matched for age and gender**.**

- **Results**

A significant increase of MDA concentration was found in both haloperidol and atypical antipsychotics treated patients, compared with the control group, suggesting an increased lipid peroxidation process. Also, the specific activity of GPX was decreased in both typical and atypical antipsychotics treated patients, suggesting an increased oxidative status. However, the activity of SOD was increased in the haloperidol group, compared to the controls. Additionally, a decrease in the specific activity of SOD was reported in the atypical (quetiapine and olanzapine) antipsychotics group.

- **Conclusions**:

1. We found elevated levels of MDA and a decreased activity for the antioxidant enzymes in the serum of chronically medicated schizophrenic patients regardless of their treatment type.
2. However, in the case of the atypical antipsychotics the oxidative stress could be an indicator of illness severity, since quetiapine and olanzapine are formally indicated for a specific group of schizophrenic patients, whose symptoms are refractory to other neuroleptics.
3. Our results could suggest that typical and atypical antipsychotics may not have a direct regulatory effect on oxidative stress in patients with schizophrenia.
4. Still, it is possible that the alterations of the antioxidant enzymes and the increased lipid peroxidation products from this study may reflect the pathological process of the disease.

## I.3 Understanding the correlations between the scores of PANNS/AIMS scales and some oxidative stress markers in treated schizophrenic patients

### A. Background

Considering that oxidative stress is a factor that can be corrected, future studies that would clearly identify the etiologic relation between antioxidant deficiencies and schizophrenia, may provide prophylactic treatments, as well as new treatment schemes in addition to available antipsychotic schemes.

This also emphasizes the possible importance of nutrient antioxidant supplementation to support the enzymatic defense system. However, despite the fact that the use of agents which modulate oxidative stress represents an exciting opportunity for schizophrenia prevention and treatment, future studies also need to carefully determine which antioxidants, at what dosages and in what combinations will have the greatest therapeutic benefit with the least risk, considering the importance of free radicals in many biological reactions.

In this way, we consider that it was important to analyze if the size of oxidative stress correlates with intensity of the symptoms in schizophrenia measured by some specific psychometric tools that has also clinical and scientific purpose, PANNS (The Positive and Negative Syndrome scale) and AIMS (Abnormal Involuntary Movement Scale)

As we mentioned earlier, oxidative stress is increasingly viewed as potentially important in the pathophysiology of schizophrenia, although the majority of the results regarding this subject seem to be a little bit contradictory. So, we tried to find if there could be some correlations between the specific activities of the most important antioxidant enzymes (superoxide dismutase and glutathione peroxidase) and a lipid peroxidation marker (malondialdehyde) and the scores of PANSS/AIMS scales.

In recent years, oxidative stress has drawn increasingly more attention of scientists, and many studies in the medical and biological fields have focused on the mechanisms underlying this process, taking into account the increased significance of oxidative stress in many neuropsychological diseases, including schizophrenia (Halliwell and Gutteridge, 2007). Oxidative stress is a biochemical event, which consists of the disruption of the oxidant/antioxidant balance, the antioxidant component exceeding its defense capacity (Sies, 1997).

Schizophrenia is a debilitating disease characterized by structural and functional changes. However, its pathogenesis is still largely unclear. The involvement of oxidative stress in the pathology of schizophrenia has been speculated by a series of researchers, many studies identifying a change in the balance between pro-oxidant and antioxidant factors in patients with cognitive diseases (Ciobica et al., 2011, Kunz et al. 2008, Martins et al., 2008). Presently, it is thought that, at least in part, the neuropathological changes in this disease may be triggered by the mechanisms associated to oxidative stress (Yao et al., 2001). Compared to other tissues, the brain has the highest percentage of unsaturated fats, which makes its cells potentially more vulnerable to the attack of free radicals (Ciobica et al., 2012, Padurariu et al., 2013). Moreover, susceptibility to free radicals is amplified by the presence at this level of an increased. number

of mitochondria, of neurotransmitter biomolecules rich in electrons, as well as a high level of oxygen (Baloyannis et al., 2006).

Most theories regarding the role of oxidative stress in schizophrenia refer to its effect on polyunsaturated fats of the neuronal membrane. Cell membrane, which has a high content of unsaturated fatty acids, plays a protective, anti-inflammatory role and indirectly an antioxidant role, favoring the physiological processes of defense against free radicals, as well as a significant role in cell signaling. In the case of increased oxidative stress, it is possible that an excess of free radicals will alter neural signal transduction and the processing of information with central effect (Herken et al., 2001, Pazvantoglu et al., 2009).

**B. Published article in this field**

Most studies that have analyzed this theory have identified a reduction in the antioxidant system activity that determines the increase of oxidative stress and which, in its turn, contributes to membrane deficits also observed in persons with this disorder (Mahadik et al., 2003). We previously showed similar aspects in the case of schizophrenic patients treated with various antipsychotics, reporting a decrease in antioxidant enzyme level, as well as an increase of the level of lipid peroxidation (Padurariu et al., 2010).

**Romeo Dobrin**, Irina Dobrin, Cristinel Stefanescu, Alin Ciobica, Ionela Lacramioara Serban, Emil Anton - Direct Correlation Between The Scores Of PANSS/AIMS Scales And Some Oxidative Stress Markers In Treated Schizophrenic Patients. Archives of Biological Sciences Belgr. 66(4), 1559-1565, 2014. **IF 2014=0,718**

**Introduction**

In schizophrenia a reduction of the specific activity of antioxidant enzymes, such as SOD (superoxide dismutase), glutathione peroxidase (GPX) or catalase, but also of nonenzymatic antioxidants (albumin, bilirubin, uric acid) has been observed (Wood et al., 2009, Dadheech et al., 2008), as well as an increase in the markers of lipid peroxidation (malondialdehyde − MDA) (Ciobica et al., 2011). In this way, in our study from 2014, we examined some enzymatic antioxidant factors (SOD and GPX), and a product of the lipid peroxidation processes (MDA), in order to assess oxidative stress in patients with cognitive disease.

In the present study, we examined some enzymatic antioxidant factors (SOD and GPX), and an end product of the lipid peroxidation processes (MDA), in order to assess oxidative stress in patients with cognitive disease. Our objective was to study the possible correlation between the scores of specific scales such as PANSS/ AIMS and the level of oxidative stress markers.

**Material and methods**

Regarding the methodology used for this study we decided to use 45 subjects, among which 39 were patients with schizophrenia and 6 matched age controls were selected from the patients of the “Socola” Clinical Hospital of Psychiatry, Iasi. The patients were diagnosed with schizophrenia according to DSM-IV criteria and aged between 18 and 60 years. The duration of the disease was of at least 5 years, with each patient receiving antipsychotic treatment for at least 2 years. The study complied with the provisions of the Declaration of Helsinki, while the biochemical determinations performed as before (Stefanescu et al., 2012). In order to study the connections between schizophrenic pathophysiology and oxidative stress, we decided to apply

Pearson's Correlation Test between PANSS/AIMS scales and the main markers of determined oxidative stress for all groups of patients (treated with haloperidol, olanzapine, risperidone and quetiapine).

**Results**

When we statistically analyzed the results based on the Pearson's test, we observed statistical significance such as: SOD vs. PANSS ($r = 0.463$, $p = 0.005$) (Fig. 1), GPX vs. PANSS ($r = 0.59$, $p = 0.0001$) (Fig. 2) and MDA vs. PANSS ($r = -0.496$ – inversely proportional correlation, $p = 0{,}002$) (Fig. 3). In the case of the AIMS correlation with the specific activity of SOD ($r = -0.624$, we observed an inversely proportional correlation, $p = 0.0001$) (Fig. 4), GPX ($r = -0.507$ – inversely proportional correlation, $p = 0.002$) (Fig. 5), and with the level of MDA ($r = 0.368$, $p = 0.03$) (Fig. 6).

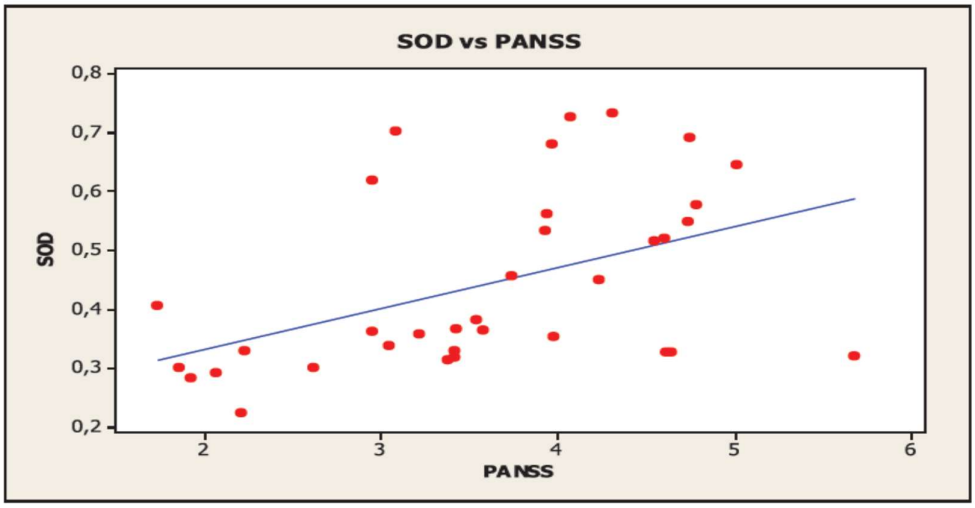


Figure. 1.1. Comparison between the dynamics of the PANSS score and the specific activity of SOD, studied by means of Pearson's correlations.

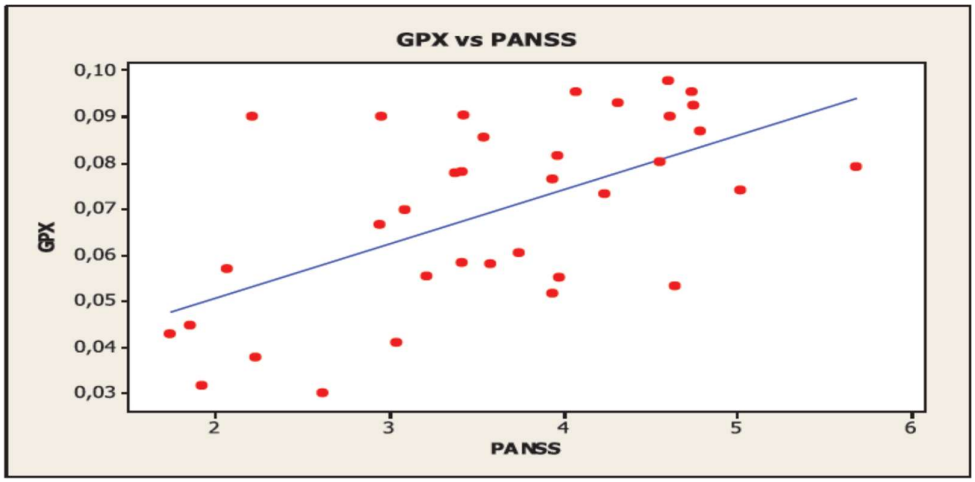


Figure. 1.2. Comparison between the dynamics of the PANSS score and the specific activity of GPX, studied by means of Pearson's correlations.

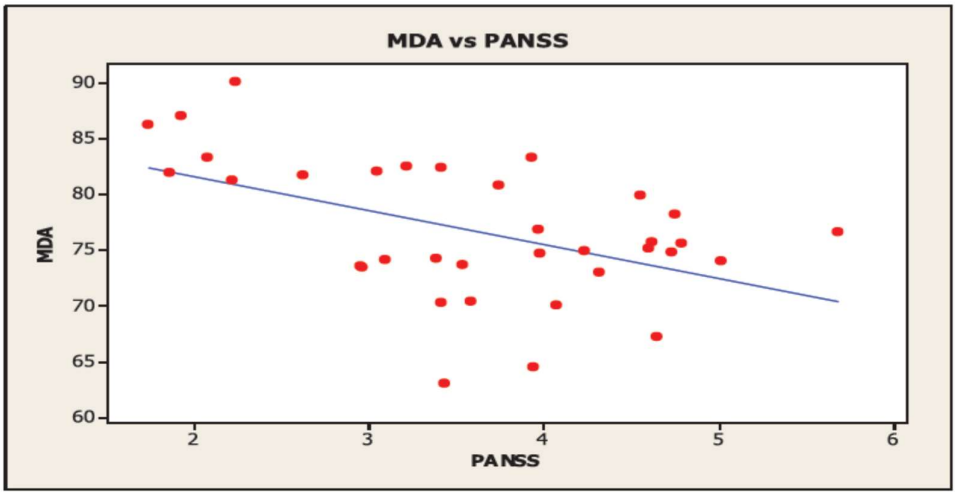

Figure. 1.3. Comparison between the dynamics of the PANSS score and the MDA concentration, studied by means of Pearson's correlations.

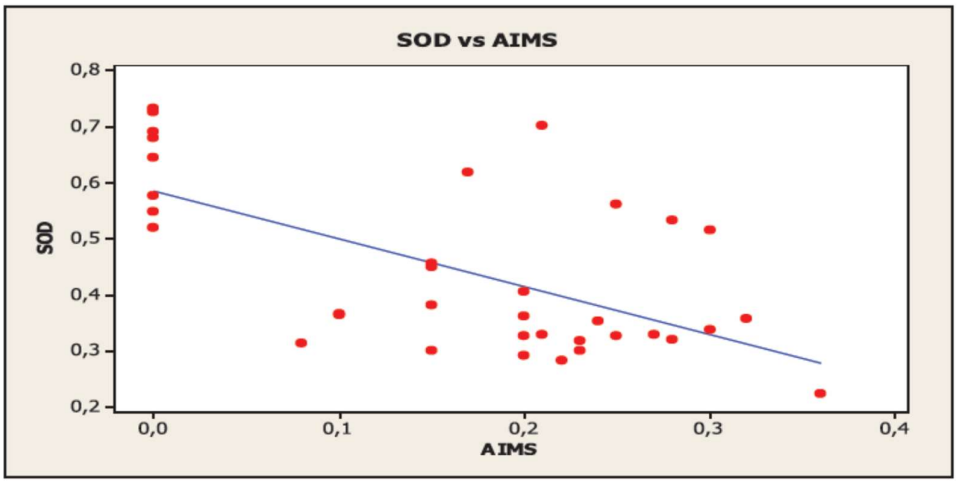

Figure. 1.4. Comparison between the dynamics of the AIMS score and the specific activity of SOD, studied by means of Pearson correlations.

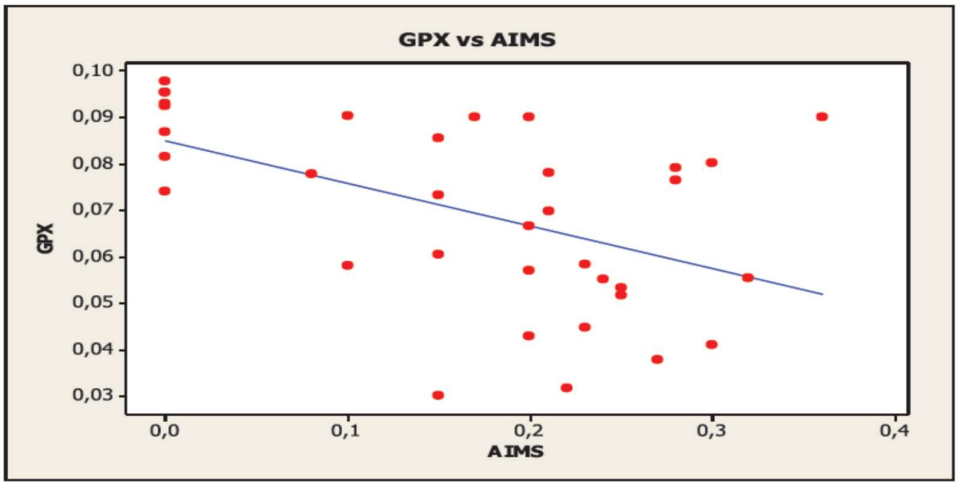

Figure. 1.5. Comparison between the dynamics of the AIMS score and the specifc activity of GPX, studied by means of Pearson correlations.

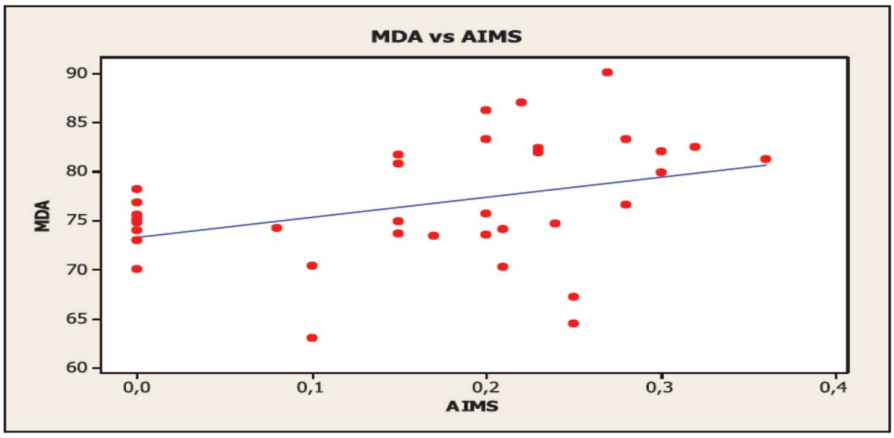

Figure. 1.6. Comparison between the dynamics of the AIMS score and the MDA concentration, studied by means of Pearson correlation

**Discussions**

Despite a significant number of articles referring to the implications of oxidative stress in psychiatric deficiencies, schizophrenia is one psychiatric disease about which very little is known with regards to the implications of oxidative stress and this aspect include differences between treated and untreated patients and the effects of treatment with typical vs. atypical antipsychotics on oxidative stress. These aspects might be due to different analyzed areas (for example tissue or blood), the different type of treatment or disease duration (Parikh et al., 2003, Raffa et al., 2009, Miljevic et al., 2010).

Considering the presented aspects, some authors suggested the use of antioxidants for the amelioration of schizophrenic symptomatology. Zhang et al. (2001a) proved in two different studies that application of a Ginkgo biloba extract along with the classical treatment with haloperidol augmented the efficiency of antipsychotics and reduced some extrapyramidal side effects. Moreover, the supplementation with Gingko biloba led to an improvement of Scale results for the negative and positive symptoms, also generating a decrease in the activity specific to SOD (Zhang et al., 2001b).

The use of essential polyunsaturated fatty acids has also been suggested, having in mind the disorders of the membrane phospholipid metabolism that occur in schizophrenic patients (Mahadik et al., 2001). Essential polyunsaturated fatty acids, such as arachidonic acid, eicosapentaenoic acid, docosapentaenoic acid, docosahexaenoic acid, and their association with different psychopathological aspects has been reported both in patients who received antipsychotic medication and those who have never been treated, immediately after the occurrence of the first psychotic episode (Arvindakshan et al., 2003).

Some authors reported a significant correlation between the severity of symptoms and the levels of arachidonic and docosahexaenoic acids in schizophrenia (Bitanihirwe et al., 2011). These levels are influenced at turn by and patient's lifestyle and diet. Moreover, it was revealed that a combination of eicosapentaenoic/docosahexaenoic acid and vitamins C and E led to a significant reduction in schizophrenic psychopathology, suggesting possible use of these supplements in long-term management of schizophrenia (Arvindakshan et al., 2003).

In addition, the use of alpha-tocopherol was mentioned in some studies, especially for the treatment of tardive dyskinesia that might appear as a side effect of longterm treatment with antipsychotics (Berger et al., 2007).

**Conclusions**

There are indications that the correlations between the parameters of oxidative stress status (SOD, GPX and MDA) and the psychometric scales PANSS and AIMS point to a connection between the psychopathology of schizophrenia and the central oxidative stress status but we do not know quite in what way these two phenomenon are bound. It seems that these composites would have special importance at the central level and in behavioral progress. At the same time, there are theories that claim that this deficiency might have existed before the occurrence of the psychotic episode, even since the embryonic stages of growth. Taking into consideration the crucial role of membrane phospholipids in the transmission of the signal from neurotransmitters or growth factors through the receiver, one may also speak of their implication in information processing of schizophrenic patients.

## I.4: The Influence of Spiperone on Oxidative Stress and Memory

**A. Background**

As mentioned earlier, one important feature of the schizophrenia is the cognitive disturbances that affects the professional and social life of the individual. Memory is often altered in schizophrenic patients. Also, very useful in understanding the effect of antipsychotics on memory and on oxidative stress are animal models of schizophrenia. Spiperone, a potent D2-like receptor antagonist, is also a typical antipsychotic belonging to the butyrophenone chemical class, which is licensed for clinical use in some countries for the treatment of schizophrenia. In addition, spiperone is also most of the times cited as a derived of the most common used haloperidol.

However, very few studies in the literature used spiperone for the blockade of the dopaminergic system. In this way, considering these very few information about the effects of spiperone on memory processes and also on oxidative stress status, we decided to study the effects of spiperone (0.4 mg/kg body weight) pre-testing intraperitoneally administration in normal rats on spontaneous alternation behavior and number of arm entries in Y-maze task and the latency time in the passive avoidance performance. Also, we were interested in seeing the effects of spiperone administration on the oxidative stress status from the hippocampus, by assessing two antioxidant enzymes: superoxide dismutase-SOD and glutathione peroxidase-GPX, as well as a lipid peroxidation marker: malondialdehyde-MDA

Regarding the effect of antipsychotics on memory function, several studies actually investigated these effects, with several controversial results, stating both positive and negative effects (Meltzer, 1999, Eitan, 1992).However, very few studies used the specific D2 antagonist spiperone for the blockade of the dopaminergic system. In this way, most of the studies regarding spiperone were connected to the so-called spiperone binding assay on the dopaminergic receptors or in even fewer cases in studying the effects of some neuroleptic ameliorative administration on the memory process, in different combinations after a phencyclidine– induced model of schizophrenia (Schroeder, 2000).

It seems that there are still some controversies regarding the effects of spiperone on memory processes, since some authors reported an ameliorative effect of his administration (Muakkassah-Kelly, 1983), while in other situations it seems to block the mechanisms implicated in memory and especially learning processes (Pogun, 1992). In addition, oxidative stress is also cited for its implications in the pathophysiology of schizophrenia (Ciobica, 2011). Moreover, the effects of spiperone administration on the oxidative stress status are not completely understood to this date. In this way, in some studies spiperone abolished the neuroprotective effect of cabergolinein cortical neurons (ODAKA, 2014). Moreover, the addition of spiperone alone had no toxic influence in the presence or absence of $H_2O_2$ , suggesting that a receptor-mediated mechanism is involved in the survival-promoting effect of cabergoline. In contrast, Zheng et al. showed that spiperone significantly decreased the production of the nitric oxide in lipopolysaccharide and adenosine 5'-triphosphate (ATP)-stimulated microglia cells, primary microglia and primary astrocyte cultures (Zheng, 1994).

**B. Published article in this field**

Our interest in this domain materialized with the publication of a study in 2014 regarding the effects of spipepirone administration on oxidative stress status.

**Romeo Dobrin**, Alin Ciobica, Elena Toader, Vladimir Poroch, The influence of spiperone on oxidative stress and memory, Revista de chimie. 2016; 67(9): 1778-1782. ISSN: 0034-7752 , **IF 2016 = 1.232**

**Objective**

In this way, considering these very few information about the effects of spiperone on memory processes and oxidative stress status, we decided to study the consequences of pre-testing intraperitoneally spiperone administration in normal rats on spontaneous alternation behavior and number of arm entries in Y-maze task and the latency time in the passive avoidance performance, as well as the effects of spiperone administration on the oxidative stress status from the hippocampus.

Moreover, we were interested in studying if there is a correlation between the behavioral parameters we determined in Y maze or passive avoidance tasks and the levels of the oxidative stress markers which we determined (two antioxidant enzymes: superoxide dismutase-SOD and glutathione peroxidase-GPX, as well as a lipid peroxidation marker: malondialdehyde-MDA), as a result o spiperone administration.

**Material and methods**

Regarding the methodology and material used to perform this experiment, we used adult male Wistar (n = 20) rats, weighing 200- 250 g at the start of the experiment, were housed in groups of five animals per cage and kept in a room with controlled temperature (22o C) and a 12:12-h light/dark cycle (starting at 08:00 h), with food and water ad libitum. The animals were treated in accordance with the guidelines of animal bioethics from the Act on Animal Experimentation and Animal Health and Welfare Act from Romania and all procedures were in compliance with the European Communities Council Directive of 24 November 1986

(86/609/EEC). This study was approved by the local Ethics Committee and also efforts were made to minimize animal suffering and to reduce the number of animals used.

We administered spiperone was performed in a dosage of 0.4 mg/kg body weight, intraperitoneally (i.p.), 30 min before Y maze and also 30 minutes before acquisition phase in passive avoidance. Short-term memory was assessed by spontaneous alternation behavior in the Y-maze task. The Y-maze used in the present study consisted of three arms (35 cm long, 25 cm high and 10 cm wide) and an equilateral triangular central area. The rat was placed at the end of one arm and allowed to move freely through the maze for 8 min. An arm entry was counted when the hind paws of the rat were completely within the arm.

Spontaneous alternation behavior was defined as entry into all three arms on consecutive choices. The number of maximum spontaneous alternation behaviors was calculated as total number of arms entered minus 2 and percent spontaneous alternation was calculated as (actual alternations/maximum alternations) × 100. Spontaneous alternation behavior is considered to reflect spatial working memory, which is a form of short-term memory.

In brief, a step through type passive avoidance apparatus (Coulbourn Instruments) consisting of two compartments (25×15×15 cm high), one illuminated and one dark, both equipped with a grid floor was used. The two compartments were separated by a guillotine door. In the acquisition trial, each rat was placed in the illuminated compartment; when the animal entered the dark compartment, the door was closed and an inescapable foot shock (0.3 mA, 5 s) was delivered through the grid floor. The rat was removed after receiving the foot shock and was placed back into the light compartment. The door was again opened 30 s later to start the next trial. The training continued until the rat stayed in the light compartment for a 120-s period on a single trial. The rats were given 3–5 trials and trained to avoid punishment (remain on shock-free zone). After 24 h, each rat was placed in the light compartment and the step through latency was recorded until 300 s had elapsed (retention trial). The step-through latency in the retention trial was used as the index of retention of the training experience. Longer retention latencies were interpreted as indicating better retention of the training experience [16].
Tissue collection.

After the behavioral test, all rats were anesthetized, rapidly decapitated and the whole brain was removed. The hippocampi were then collected. Each of the samples was weighed and homogenized with a Potter Homogenizer coupled with Cole-Parmer Servodyne Mixer in bidistilled water (1g tissue/10mL bidistilled water). Samples were centrifuged 15 min at 3000 rpm. Following centrifugation, the supernatant was separated and pipetted into tubes.

Malondialdehyde (MDA) concentrations were determined by thiobarbituric acid reactive substances (TBARs) assay. 200µL of supernatant was added and briefly mixed with 1 mL of trichloroacetic acid at 50%, 0.9 mL of TRIS-HCl (pH 7.4) and 1 mL of thiobarbituric acid 0.73%. After vortex mixing, samples were maintained at 100OC for 20 min. Afterwards, samples were centrifuged at 3000 rpm for 10 min and supernatant read at 532 nm. The signal was read against an MDA standard curve and the results were expressed as nmol/mg protein.

Superoxide dismutase (SOD) activity was measured by the percentage reaction inhibition rate of enzyme with WST-1 substrate (a water soluble tetrazolium dye) and xanthine oxidase using a SOD Assay Kit (Fluka, product number: 19160) according to the manufacturer's instructions. Each endpoint assay was monitored by absorbance at 450 nm (the absorbance wavelength for the colored product of WST-1 reaction with superoxide) after 20

min of reaction time at 37°C. The percent inhibition was normalized by mg protein and presented as SOD activity units.

Glutathione peroxidase (GPX) activity was measured using the GPX cellular activity assay kit CGP-1 (Sigma Chemicals). This kit uses an indirect method, based on the oxidation of glutathione (GSH) to oxidized glutathione (GSSG) catalyzed by GPX, which is then coupled with recycling GSSG back to GSH utilizing glutathione reductase (GR) and NADPH. The decrease in NADPH at 340 nm during oxidation of NADPH to NADP is indicative of GPX activity (Mocanu 2015, Scrobota, 2015).

**Results**

We observed that in the passive avoidance task, there was a significant decrease of the latency time (long-term emotional memory), on both 24 ($F(1.18) = 23$, $p < 0.0001$) and 72 h ($F(1.18) = 14$, $p = 0.001$), as a result of spiperone administration, when compared to the control group. However, in the spatial task of the Y-maze, no significant differences between groups was noticed on the spontaneous alternation (immediate working memory) ($F(1.18) = 2$, $p = 0.1$), as well as the number of arm entries (locomotor activity) ($F(1.18) = 0.5$, $p = 0.4$) (Table 1.2).

Table 1.2. Values for behavioral parameters in control and pre-test spiperone treated rats (Mean ± Sem)

| | Groups | | |
|---|---|---|---|
| **Parameter** | **Control** | **Spiperone** | **P value** |
| MDA (nmol/mg protein) | 57.14±4.46 | 259.83±17.82 | <0.0001 |
| SOD (U/mg protein) | 0.9±0.08 | 0.52±0.038 | 0.004 |
| GPX (U/mg protein) | 0.31±0.029 | 0.2±0.02 | 0.0007 |

MDA= malondialdehyde; SOD=superoxide dismutase; GPX= glutathione peroxidase

When we administered spiperone, we noticed a significant increase of oxidative stress, as revealed by a very significant increase in the MDA concentration ($F(1.18) = 144$, $p < 0.0001$), which is an important marker of the lipid peroxidation processes. Moreover, the D2 receptor antagonist spiperone significantly decreased the specific antioxidant activity of both SOD ($F(1.18) = 10$, $p = 0.004$) and GPX ($F(1.18) = 16$, $p = 0.0007$) (Table1. 3).

Table 1.3. Values for oxidative stress parameters in temporal lobe from control and pre-test spiperone treated rats (Mean ± Sem)

| | Groups | | |
|---|---|---|---|
| **Parameter** | **Control** | **Spiperone** | **P value** |
| **Step-through passive avoidance** | | | |
| First-day (24 h) latency test (s) | 300±0 | 164.77±44.79 | <0.0001 |
| 72 h latency test (s) | 260.69±30.91 | 131.50±53.39 | 0.001 |
| **Y maze task** | | | |
| Spontaneous alternation (%) | 80.74±6.53 | 73.27±6.39 | 0.1 |
| Number of arm entries | 13.59±0.78 | 14.09±0.94 | 0.4 |

Also, Pearson's correlation coefficient and regression analysis, significant correlations were found in the case of first day (24 h) latency in the passive avoidance vs. the main markers of the oxidative from the hippocampus, which we determined in this study, as in the case of MDA (n = 20, r = -0.637, p = 0.003), or in the case of the first day latency vs. SOD (n = 20, r = 0.703, p = 0.001) or first day latency vs. GPX (n = 20, r = 0.398, p = 0.043) (fig. 1). The same applies for the latency time in the passive avoidance task after 72 hours vs. MDA (n = 20, r = -0.590, p = 0.0036), SOD (n = 20, r = 0.405, p = 0.041) and also GPX (n = 20, r = 0.422, p = 0.0043) (figure 1.7).

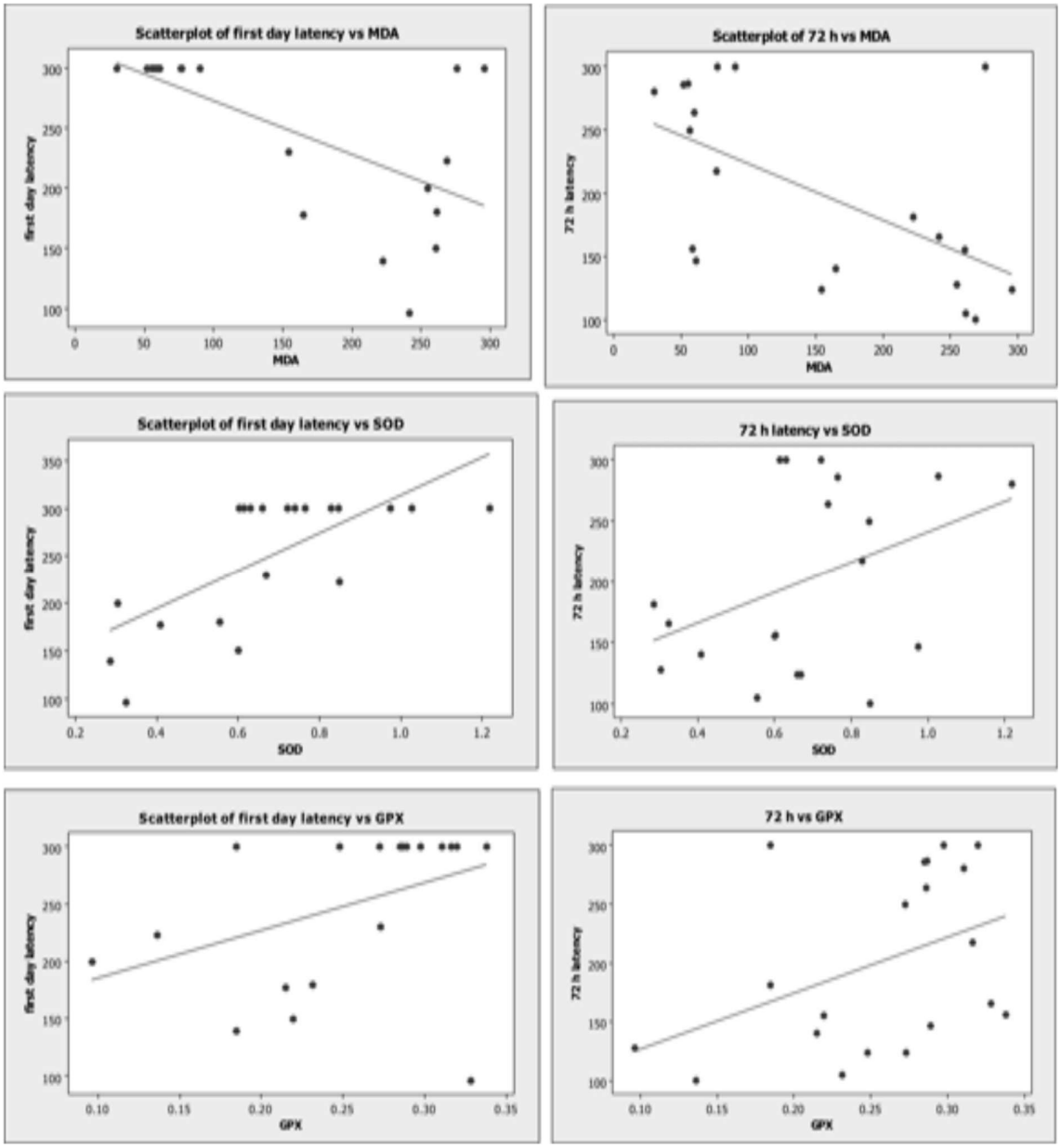


Figure 1.7. The correlations between the behavioral parameters of the passive avoidance (24 h and 72 latency time) vs. the main markers of the oxidative from the hippocampus (superoxide dismutase-SOD, glutathione peroxidase-GPX and malondialdehyde-MDA).

**Discussions**

In this way, it seems that the systemic administration of drugs that stimulate dopamine D2 receptors has been reported to improve cognitive functions in rats (Stuchlk, 2007) and humans (Kimberg,1997), while the blockade of dopamine D2 receptors impaired those functions in rats (Padurariu, 2013) and humans (Mheta, 1999). In our study we found that spiperone administration, a dopamine D2 antagonist, was associated only with longterm memory dysfunction in rats.

Thus, we report here that pre-test intraperitoneally administration of spiperone resulted in significant decrease of the latency time (longterm emotional memory) in the passive avoidance task. However, no significant modifications were observed when spiperone was administrated before Y maze task.

Our results are suggesting reduced effect of spiperone on D2 receptors involved in spatial efficiency and motor activity in the Y maze task, and a predominant influence on D2 receptors implicated in consolidation of passive avoidance memory. In contrast, other studies have demonstrated that another D2 receptor antagonist, sulpiride, intraperitoneally administrated in rats before the active place avoidance task, resulted in decreased locomotor activity and affected spatial behavior (Tarantino, 2019) while systemic post-test sulpiride administration enhanced retention in both the hidden and visible platforms of the Morris water maze (Setlow, 2000).

In addition, the passive-avoidance test has been used previously to evaluate the effects of the antipsychotic drug haloperidol, which is also a D2 receptor antagonist, on learning and memory function in rodents, with the Japanese group of Ichihara et al. founding that pre-test haloperidol administration did not impair the passive avoidance response at doses lower than those inducing motor disturbances (e.g. sedation) during the training session.

Moreover, it seems that all these contrasting findings could be related to different binding characteristics of sulpiride and spiperone on D2 receptors (Florijn, 2007). On the other hand, our previous studies also showed that the administration of pergolide, an agonist for both D1 and D2 receptors, resulted in a decreased oxidative stress status in the temporal lobe of a 6-OHDA rat model of Parkinson's disease, as demonstrated especially by a significant reduction in the MDA levels, which was also correlated with an improvement of the spatial memory tested in specific behavioral tasks such as radial-8-arm maze and Y-maze (Ciobica, 2011).

In our present study, the intraperitoneal administration of spiperone in normal rats resulted in a significant increase for the oxidative stress status of the hippocampus, as demonstrated by the increase in the concentration of MDA, a lipid peroxidation marker, and the significant decrease for the specific activities of the main antioxidant enzymes SOD and GPX. As already discussed, previous studies on the underlying mechanisms mediated by D2 receptors and related to the oxidative stress manifestations have revealed that the stimulation of D2 receptors by cabergoline is suppressing the ERK and p38 signaling pathways in cortical neurons. Also, spiperone abolished the neuroprotective effect of cabergoline, while the addition of spiperone alone had no toxic influence in the presence or absence of $H_2O_2$ (Odaka, 2014).

As also mentioned before, it was demonstrated that spiperone could exert protective effects against inflammation mediated neurodegeneration, mainly through a decreased the production of nitric oxide in microglia cells.

In this way, spiperone attenuated the expression of inducible nitric oxide synthase and proinflammatory cytokines such as interleukin-1beta and tumor necrosis factor-alpha in microglia cells. The different dependency on receptor mediated mechanisms may be attributed to differences in the cell-types used, cortical neurons, mesencephalic neurons/cell line or microglia cells (Zheng, 2008).

Also, since we suggested in this paper a possible connection between oxidative stress modifications and memory consolidation, especially in the less known context of the spiperone administration in rats, we should also mention the increased relevance of the oxidative stress status in the main neuropsychiatric disorders, starting with dementia, as our group previously demonstrated in several different instances (Padurariu, 2013).

Even more than that, it was previously shown that oxidative stress could be quite important also in the schizophrenic pathology (Paduraru, 2010) and there is also a significant correlation between the main markers of the oxidative stress status and some specific scales for schizophrenia such as PANSS and AIMS (Dobrin, 2014).

In this context, it is also worth mentioning the increased awareness from the current literature in understanding the cognitive and memory deficits in schizophrenia, ranging from alterations in memory, attention and motor skills deficiencies to a significantly affected intelligence (O'Carroll, 2000)

In fact, similar aspects regarding a possible connection between the oxidative stress status and superior cognitive functions in most of the neuropsychiatric disorders were also demonstrated (including by the results of our group) in PD (Kimberg, 1997) depression (Stefanescu, 2012) or even autism (Ciobica, 2015).

In addition, the biological importance of this connection is demonstrated by its presence even on the lower scale of evolution models, such as in species like Drosophila, as in the work of Haddadi et al. group which demonstrated quite recently the importance for the accumulation of oxidative damage and reduction of antioxidants in aging and functional senescence, by showing important correlations between consolidated forms of olfactory memory and a decrease in the antioxidant enzymes such as catalase and SOD or reduced glutathione level, as well as by an increase in the lipid peroxidation and reactive oxygen species (ROS) concentrations in the brain of the aforementioned Drosophilla age-related memory impairment model (Haddadi, 2014).

These consistent results regarding the connections that might exist between memory consolidation and oxidative stress status could be explained by the fact that the brain has low levels of antioxidants, as compared to the other organs, while on the other hand it is very rich in polyunsaturated fatty acids and catecholamines (important oxidizable substrates) and also it one of the most important oxygen consumers in the body. Of course, all these data are suggesting the potential benefits of the antioxidant therapy.

However, most of studies are generally controversial in regard to the usefulness of these drugs in most of the neuropsychiatric disorders. Also, the usage of some antioxidants for these superior functions-related disorders prevention or treatment is extremely controversial, given also some additional speculated side effects of these compounds (Padurariu, 2013).

Still, there are data such as the one of Zhangh et al. from example, which showed in two different studies that adding a Ginkgo biloba extract to classical haloperidol treatment could result in better scores in the Scales for the Assessment of Positive and Negative Symptoms

while also enhancing the effectiveness of the antipsychotic and reducing some extrapyramidal side effects (Zhang, 2001).

So, in summary, we found that the administration of spiperone, a D2 receptor antagonist, could impair memory processes in normal rats, while also generating increased levels of lipid peroxidation markers such as MDA and decreased enzymatic antioxidants in hippocampus.

Moreover, we found significant correlations between the behavioral parameters we determined in the passive avoidance tasks and the levels of all oxidative stress markers which we determined, because of spiperone administration, suggesting that oxidative stress could be related to some memory deficits induced by spiperone in normal rats.

However, present studies are being performed in our lab, to see the effects of spiperone administration in a ketamine-induced rat of schizophrenia (on some specific memory-related behavioral tasks and on oxidative stress levels). Also, the relevance of some synthetic SOD and GPX mimetics is right now tested in our lab to see if it can reverse the spiperoneinduced memory impairments. In addition, we are planning to look to more specific areas of the brain in the future for the oxidative stress status modifications, such as hippocampus, amygdala or the striatum.

Regarding the latest developments on the connection between oxidative stress and schizophrenia, lately it is believed that the mechanism of oxidative stress damage may be a common pathway of some psychiatric disorders, but it was mostly studied in schizophrenia. In order to have a better understanding of the disease that may lead to better treatment options, investigating oxidative stress role in schizophrenia may be an easy way.

Considering that, there are many aspects of oxidative stress to study in schizophrenia including establishing reliable biomarkers either peripheral or central, understanding how oxidative stress markers change with the course of disease, or with treatment and also if there is real clinical benefit in giving antioxidants to schizophrenic patients, what kind of antioxidants may work or if antioxidants may have preventing properties in the first place.

Research in the area of oxidative stress in schizophrenia is fairly extensive, but the exact cause of oxidative stress increase in schizophrenia is not known. Some studies suggest that disturbances in the metabolism of fatty acids and phospholipids may partially explain the increase in oxidative stress status in the brain of these patients (Wang, 2020).

A decrease in the activity of some antioxidant enzyme such as paraoxonase 1 diminishes the antioxidant defence against lipoperoxidation in deficit schizophrenia, as it was reported by Matsumoto and his colleagues (Matsumoto, 2020). A decrease in the antioxidant activity may predispose to immune dysregulation and neurotoxic effects induced by immunologic, inflammatory, oxidative and nitrosative reactions.

Also, oxidative stress may be more relevant in some types of schizophrenia. For instance, a recent article published in 2020 after a multiple multicentric research regarding deficit syndrome in schizophrenia report an umbalance between antioxidant and prooxidant activity with increase in oxidative stress. Authors found that this particular group of patients showed an increase in advanced oxidation protein products and a decrease of paraoxonase 1 activity.

Also, when compared with non-deficit schizophrenia they found an increased oxidative stress toxicity biomarkers and a decrease of antioxidants ratio in deficit schizophrenia (odds ratio = 3.15, $p < 0.001$). The intensity of symptoms in schizophrenia is also found to be

positively correlated with an increased oxidative neurotoxicity that exceeds the protective brain mechanisms (Maes, 2020).

Concerning the central biomarkers, there are some studies that have found a significant decrease of the GSH, GpX and glutathione reductase concentration in the brain of schizophrenia patients when compared to the level of these enzymes found in the brains of control group, which actually correlates with the peripheral level (Radonjić, 2010, Morris, 2020). Also, on the other hand, there are reports that found only a small reduction in these enzymes or even an increase of GSH brain level (Terpstra, 2005). The heterogeneity of these evidences suggests indeed changes of oxidative status in schizophrenia, but the results have to be carefully interpreted.

Still, there is no clear evidence that peripheral oxidative stress markers are a reflection of to the central oxidative status. We can, at most, obtain correlations between peripheral and central oxidative biomarkers without knowing if the peripheral oxidative status is a reflection of the central oxidative status.

**Conclusions**

No significant correlations were obtained in the case of the behavioral parameters in the Y maze task (spontaneous alternation percentage and number of arms entries) vs. the oxidative stress makers, except for the spontaneous alternation vs. GPX ($n = 20$, $r = 0.466$, $p = 0.0039$) (Figure 1.7).

These data could suggest that the increase we showed in the oxidative stress status could be correlated the memory deficits observed because of spiperone administration.

As mentioned before, the studies investigating the role of dopamine D2 receptors in the cognitive functions such as long-term memory, working memory and locomotor activity are quite limited.

# SECTION I: CHAPTER 2
# Oxidative stress markers and the management of alcoholism

**Introduction**

Alcohol is the most widely consumed drug world - wide and its levels of mortality and morbidity are increasing constantly, considering that alcohol abuse has been associated with a vast range of pathologic conditions. Alcoholism is a medical, social and personal condition that affects a wide range of individuals, disregarding of financial status, age, race or profession. Our focus with this disorder is justify by the multiple complication associated with alcoholism but also with the difficulty to treat the condition and the high risk of relapses. Since there are only few treatments on alcoholism and also on the complications emerged from this, we investigated oxidative stress status in alcoholism.

This chapter is compiled from the work done under three publications, looking at the oxidative stress status in the early and stages of alcohol abstinence which confirmed the increased oxidative stress status in alcoholic patients (as showed by decreased antioxidant enzymes and increased lipid peroxidation). We also demonstrated a significant decrease in the oxidative stress status one week and one month following the withdrawal, as showed by a significant increase in the specific activity of SOD, as well as by a decrease in MDA levels, when compared to baseline. Still, in the case of all three markers of the oxidative stress status which we determined (SOD, GX and MDA), the levels from one week or one month of abstinence were significantly altered when compared to controls, suggesting that severe and prolonged deficiency in their levels needs longer than one month of abstinence to normalize.

As a follow up we looked into the same oxidative stress markers in the late stages of alcohol abstinence. In this way, the data we presented in this study again confirmed the increased oxidative stress status in alcoholic patients and even more importantly, we showed that there is a significant and progressive decrease in the oxidative stress status, as demonstrated by the increased levels of antioxidant enzymes and decreased rate of lipid peroxidation, when compared to baseline values. Still, in the case of all three markers of the oxidative stress status which we determined back then, the levels from one week or one month of abstinence were significantly altered when compared to controls, suggesting that severe and prolonged deficiency in their levels needs longer than one month of abstinence to normalize.

Finally, we analized systemic treatment of acohol withdrawal versus pharmacological treatment alone. Although there are numerous studies on the treatment of alcohol dependence, ranging from various schemes of pharmacologic treatment to specific psychotherapeutic approaches, the reviews and meta-analyses reveal only modest effects of these approaches, in many cases with the costs exceeding benefits. Our study shows that taking into account begavioural aspects of alcohol dependence management significantly improves the outcome, mainly in terms of maintaining abstinence and improving the quality of life. The therapeutical interventions are not only effective in improving the clinical outcome, but also costwise, which cound prove to be extremely beneficial considering that alcohol addiction is a growing public heath problem.

## 2.1 The relevance of oxidative stress status in one week and one month alcohol abstinent patients

### A. Background

Although it is now generally accepted that there is an increased oxidative stress status in alcoholics, mainly expressed through a reduction in the general antioxidant activity (a distinct feature of chronic alcohol exposure) (Lecomte, 1994) as well as by increased levels of lipid peroxidation markers such as MDA in both cerebrospinal fluid or peripheral venous blood (Shaw, 1989), the relevance of oxidative stress following alcohol withdrawal is still not understood to this date.

There are reports stating that the increased oxidative stress status from alcoholics may persist independently of the constant presence of alcohol intake, perpetuating a prooxidative condition in the body even during abstinence (Lieber, 1988). On the other side, it was demonstrated that the antioxidant defense mechanism (as represented for example by SOD, the first antioxidant enzyme in the way of the free radicals, could significantly increase after alcohol withdrawal (Lecomte, 1994).

In fact, for the specific activity of SOD, there is a multitude of controversial studies, indicating an increase following alcohol withdrawal (as mentioned before), as well as a decrease (Tsai, 1998) or modifications, irrespective of alcohol ingestion or abstinence, or the kind of treatment administered (Marotta, 1997).

### B. Published article in this field

Our interest in this field materialized by publishing an article in 2014 regarding the differences in oxidative stress status in different duration alcohol abstinent patients.

Ovidiu Alexinschi, Roxana Chirita, Alin Ciobica, Padurariu Manuela, **Romeo Dobrin**, Raluca Prepelita, Ionela Lacramioara Serban, Vasile Chirita - The relevance of oxidative stress status in one week and one month alcohol abstinent patient. Journal of Medical Biochemestry 33(3), pg. 284-290, 2014, **IF 2014 = 1,045**

### Introduction

We were particularly interested in studying the relevance of oxidative stress status in the alcohol withdrawal processes, by determining some oxidative stress markers SOD, GPX and a lipid peroxidation maker – MDA after one week and one month of abstinence, as compared to the baseline (oxidative stress status at the time of entry into the study) and a control group of subjects.

### Materials and methods

In order to investigate the status of oxidative stress in abstinent patients, in our methodology we included 33 male patients aged between 26 and 79 years (average 45.2±3.58 years), in alcohol abstinence. They fulfilled the Diagnostic and Statistical Manual of Mental Disorders IV Text Revision (DSM-IV-TR) diagnostic criteria of alcohol dependence and were scheduled to be admitted to the alcohol detoxification ward (no. II B) for further alcohol

dependence rehabilitation in the Psychiatry University Hospital, Iasi, Romania. Alcohol consumption was stopped abruptly at admission. Alcoholic patients without illicit drug use, chronic systemic disease or severe mental disorders were selected. They were all treated with diazepam or lorazepam and also supplemented with vitamins from the B group.

The detoxification ward was a restricted environment for one week to one month for the patients included in the study, who received identical meals provided by the hospital. However, only 19 from the initial 33 patients remained in the ward for one month, for the final blood collection, since most of them decided to drop out of the study (withdrawal symptoms ameliorated and discharge at patients' request or refusal to give another sample of blood). The control group (n=18) included healthy age and sex-matched subjects without known physical or psychiatric illnesses which were identified by clinical interview and routine laboratory tests. They also did not meet the diagnostic criteria of alcohol abuse or dependence in the past nor abusive alcohol consumption during the previous two months. The study was conducted according to provisions of the Helsinki Declaration and the local ethics committee approved the study. All the patients or their families signed the consent for the participation in this study. Regarding the methodology, we collected blood samples (n=33), after one week (n=33) and after one month (n=19), in the morning, before breakfast, allowed to clot and centrifuged immediately. Serum was aliquoted into Eppendorf tubes and stored at −40 °C until measurement.

**Results**

The classical biological markers of chronic alcoholism such as gamma-glutamyl transferase, aspartate transaminase and alanine aminotransferase were determined, being significantly higher in alcoholic patients at baseline, when compared to controls. Additionally, we observed, as expected that all these markers decreased significantly after 1 week and 1 month of detoxification. Regarding the specific activity of SOD, which is the first enzyme in the way of ROS, we found a significant decrease at both baseline time ($F(1.49)=54$, $p<0.0001$), as well as in the case of measurements that were taken after one week ($F(1.49)=11$, $p=0.001$) or one month ($F(1.35)=17$, $p<0.0001$) after withdrawal, when compared to our set of control subjects (Figure 2.1). Moreover, when we applied the post hoc analysis, we observed significant differences between the baseline and one week ($p<0.0001$) or baseline vs. one month groups ($p=0.003$), as well as between one week and one month groups ($p=0.003$) (Figure 2.1).

Also, in the case of the other antioxidant enzyme we determined here, GPX, we observed a significant decrease at the baseline time ($F(1.49)=7$, $p=0.01$), as well as after 1 week ($F(1.49)=9$, $p=0.004$) or one month ($F(1.35)=17$, $p=0.01$) after withdrawal, as compared to the control group (Figure 2.2).

Additionally, in this case, the post hoc analysis showed no significant differences between baseline and one week ($p=0.27$) or baseline vs. one month groups ($p=0.98$), as well as between one week and one month groups ($p=0.28$) (Figure 2.3). When we determined the serum levels of MDA, as a fundamental parameter of the lipid peroxidation processes, we observed a very significant increase in the MDA levels at baseline ($F(1.49)=13$, $p=0.004$) or one month ($F(1.35)=17$, $p=0.01$) after withdrawal, as compared to the control group (Figure 2.2).

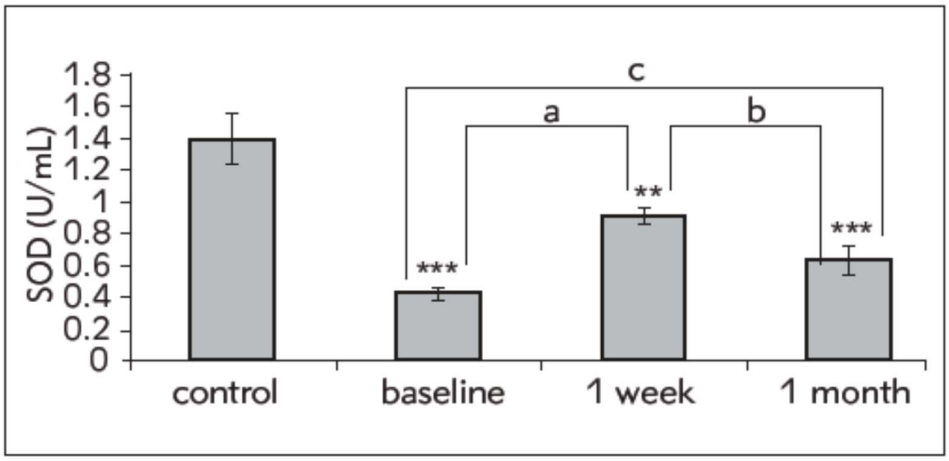


Figure 2.1. Superoxide dismutase (SOD) specific activity in the serum of control subjects, baseline and alcohol abstinent patients after one week and one month. The values are mean ± SEM (n=18 in control group, n= 33 in baseline and one week groups and n=19 in one month group). **p<0.001; ***p<0.0001. For post hoc analysis – a (baseline vs. one week): p<0.0001; b (one week vs. one month): p=0.003; c (baseline vs. one month): p=0.003.

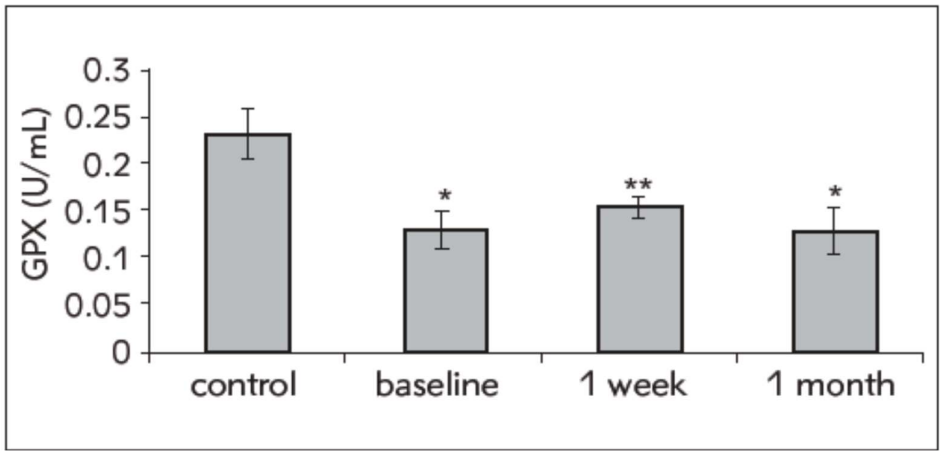


Figure 2.2. Glutathione peroxidase (GPX) specific activity in the serum of control subjects, baseline and alcohol abstinent patients after one week and one month. The values are mean ± SEM (n=18 in control group, n=33 in baseline and one week groups and n=19 in one month group). *p=0.01; **p=0.004.

Additionally, in this case, the post hoc analysis showed no significant differences between baseline and one week (p=0.27) or baseline vs. one month groups (p=0.98), as well as between one week and one month groups (p=0.28) (Figure 2.2). When we determined the serum levels of MDA, as a fundamental parameter of the lipid peroxidation processes, we observed a very significant increase in the MDA levels at baseline ($F(1.49)=13$, $p<0.0001$), when compared to control subjects (Figure 2.3). Still, the levels of MDA were normal after one week ($F(1.49)=2$, $p=0.11$) and one month ($F(1.35)=1$, $p=0.2$), since no significant differences were observed in these two groups (one month and one week), when compared to controls (Figure 2.3).Additionally, the post hoc analysis for MDA levels showed significant differences between baseline and one week determinations (p=0.019), as well as between baseline and one month (p=0.004). However, no significant differences regarding the concentrations of MDA were found between one week and one month determinations (p=0.4) (Figure 2.3).

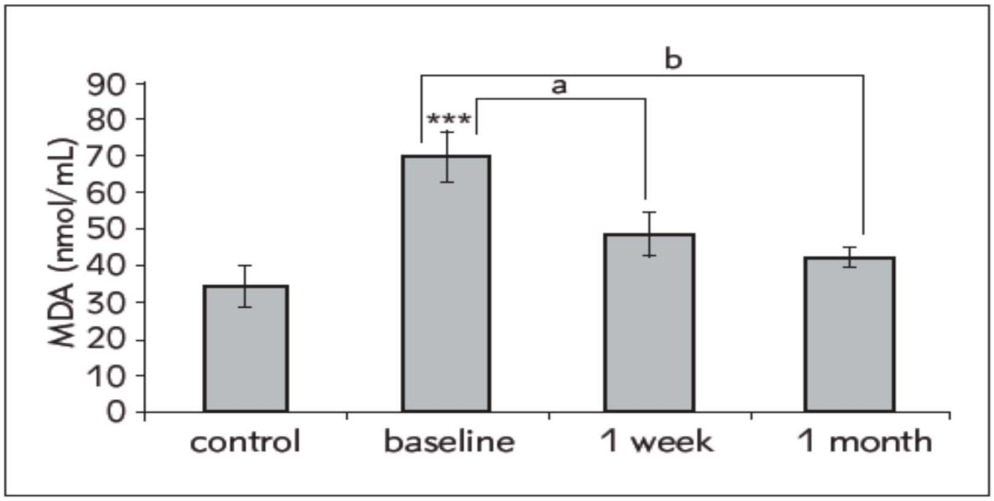


Figure 2.3. The levels of malondialdehyde (MDA) in the serum of control subjects, baseline and alcohol abstinent patients after one week and one month. The values are mean ± SEM (n=18 in control group, n=33 in baseline and one week groups and n=19 in one month group). ***p<0.0001. For post hoc analysis – a (baseline vs. one week): p=0.019; b (baseline vs. one month): p=0.004.

**Discussions**

In this way, we observed in this presented study an increased oxidative stress status in alcoholic patients and, more importantly, we demonstrated here a significant decrease in the oxidative stress status one week and one month following the withdrawal, as showed by a significant increase in the specific activity of SOD, as well as by a decrease in MDA levels, when compared to baseline. As mentioned, we demonstrated an increased oxidative stress status in the patients with alcohol dependence, considering the reduced specific activity of both SOD and GPX, completed by the significant increase in MDA, as a specific marker of the lipid peroxidation processes, when compared to our age-matched subjects. These aspects are consistent with previous reports stating that the antioxidant activity is lower in chronic alcoholics than in control subjects. This was demonstrated for the antioxidant enzymes such as SOD, GPX and CAT (Huang, 2009, Peng, 2005) but also for the classic antioxidants such as glutathione (Kannan, 2004). Also, the lipid peroxidation processes, as evaluated through the various levels of thiobarbituric acid reactive substances (TBARS), were reported to be significantly decreased in the CSF or blood of chronic alcohol consumers (Yuksel, 2005, Le Lan C 2004).

The mechanistics responsible for these effects are mainly represented by the mobilization of Fe3+ ions, acetaldehyde, as well as an alcohol-induce increase in NADPH-oxidases (Shaw, 1990, Steinberg, 1991). Also, the alcohol-inducible cytochrome P450 isoform 2E1 (CYP2E1) seems to play a very important role in the chronic alcohol consumers by generating an increased rate of ROS formation (Cederbaum, 1989, Shaw, 1989).

Regarding the withdrawal processes and relevance of oxidative stress in this matter, there are a lot of controversial results. In this way, as we previously mentioned for SOD, the first antioxidant enzyme against reactive oxygen species, both increased or decreased specific activity was reported following withdrawal.

Similarly, in the case of GPX, there are reports describing a significant decrease in its specific activity following withdrawal (Lecomte, 1994), while other authors failed to find any

significant modification in GPX during abstinence (Girre, 1990). Also, for MDA, the main marker of the lipid peroxidation processes, while several studies have found elevated levels in the serum of patients undergoing alcohol withdrawal (Yuksel, 2005, Soardo, 2005), there are also reports describing clear reductions in the various markers lipid peroxidation following alcohol withdrawal (Situnayake, 1990).

Regarding the results of this presented study, we report here a significant decrease in the oxidative stress status both one week and one month following the withdrawal, as showed by a significant increase in the specific activity of SOD, as well as by a decrease in MDA levels, when compared with those at baseline. Still, no significant modifications between the levels of GPX at baseline, one week and one month were observed.

One possible explanation for this could be the fact that SOD is the most sensitive to and the primary defense against ROS damage (Sies, 1997), exhibiting prompt compensation processes when encountering or not heavy oxidative injury. Therefore, it is possible for the compensatory modifications of GPX to be less sensible, as compared to SOD.

However, in the case of all three markers of the oxidative stress status we determined here, the levels from one week or one month of abstinence were significant.

Also, despite some opinions that in fact the so called detoxification of alcohol-dependent patients still leads eventually to a restoration of the prooxidant processes (Huang, 2009), it was also very recently demonstrated that oxidative stress could be relevant for the ameliorative processes that would actually appear after a period of abstinence in pathologies like alcoholic hepatitis (Wang, 2012). In this way, the Wang group showed that SOD, as well as glutathione levels, were increased in alcoholic hepatitis patients with abstinence, when compared to those without abstinence, while the levels of MDA were significantly decreased.

It seems that glutamatergic neurotransmission could be extremely relevant for the oxidative stress mechanisms during alcohol abstinence (Tsai, 1998). Hence, Tsai et al. demonstrated that the augmentation of this excitatory neurotransmission may lead to enhanced oxidative stress, which together with reduced inhibitory neurotransmission may contribute to the symptoms of ethanol withdrawal and be associated with its neurotoxicity.

Another important aspect could be represented by the N-methyl-D-aspartate (NMDA) toxicity, which is known as an important mechanism in the alcohol withdrawal symptoms, by increasing both ROS and nitric oxide (NO), through the upregulation of NO synthase (NOS) (Gunasekar, 1995). This could be very important, considering that lately there is an increased awareness regarding the interactions that might exist between the oxidative and nitrosative stress status in various cellular insults (Bild, 2013).

Moreover, as it was demonstrated in some animal studies that NOS inhibition resulted in an attenuation of alcohol withdrawal symptoms (Huang, 2009b, Adams, 1995). Of course, all the aforementioned aspects lead to the idea of using antioxidants in order to ameliorate damage done by alcohol consumption and abstinence.

Thus, N-acetylcysteine (NAC) was proposed for the improving of myocardial oxidative stress in alcoholic heart disease, an important pathologic condition associated with alcoholism. The protective effects of NAC could be also explained by the fact that it is required for glutathione biosynthesis, thus inhibiting ROS toxicity (Diniz, 2006).

Additionally, it was demonstrated that alcohol-induced oxidative stress could be inhibited by NAC administration (Ozaras, 2003, Seiva, 2009b). Moreover, it was showed that

in fact NAC intake and ethanol abstinence interact synergistically in order to improve the hepatic antioxidant defenses and to decrease the serum lipids concentrations (Seiva, 2009b). It was also demonstrated relatively recently (Otis, 2010) that procysteine could increase alcohol-depleted glutathione stores in the plantaris muscle of some rat models, after a period of abstinence, considering that chronic alcohol abuse may lead to a variety of skeletal muscle complications, including atrophy, altered gait and impaired mobility (Otis, 2010).

There are, of course, several limitations of our present study, including the lack of vigorous control for the diet, body mass index and alcohol concentrations consumed before withdrawal, as well as the limited number of patients used and also the relatively short period of time chosen for the observation.

Still, further studies regarding the aforementioned markers and their relevance in the present study are underway right now by our group, as well as additional experiments regarding the oxidative stress status in patients with alcohol dependence following 3, 6 or 12 months after withdrawal.

Another aspect could be represented by the fact that the patients took antioxidants such as the B group vitamins, but this could not be avoided since it represents a classical way to treat this kind of patients.

Still, all the patients took without any exceptions exactly the same combination of B1+B6 vitamins.

**Conclusions**

In summary, our present study confirmed the increased oxidative stress status in alcoholic patients (as showed by decreased antioxidant enzymes and increased lipid peroxidation). We also demonstrated a significant decrease in the oxidative stress status one week and one month following the withdrawal, as showed by a significant increase in the specific activity of SOD, as well as by a decrease in MDA levels, when compared to baseline. Still, in the case of all three markers of the oxidative stress status which we determined (SOD, GX and MDA), the levels from one week or one month of abstinence were significantly altered when compared to controls, suggesting that severe and prolonged deficiency in their levels needs longer than one month of abstinence to normalize.

## 2.2 The dynamics of some oxidative stress markers in 3, 6 and 12-months alcohol abstinent patients: possible relevance for the usage of antioxidants in alcohol withdrawal

**A. Background**

As we mentioned above, alcoholism is a chronic condition that affects multiple organs and tissues and implementing an appropriate management of it may have an overall positive impact on individual health. One possible mechanism of inducing toxicity in alcoholism may be via oxidative stress mechanism. But, while it is generally accepted that there is an increased oxidative stress status in patients with alcohol dependence, mainly expressed through a reduction in the general antioxidant activity and a significant increase of the lipid peroxidation processes (Seiva, 2009a), the exact relevance of the oxidative stress markers after the complex processes of alcohol withdrawal is still controversial.

**B. Published article in this field**

Following the results in Chapter 2.1 we looked to further research the field of oxidative stress status in alcohol by measuring oxidative stress markers in 3, 6 and 12 months alcohol abstinent patients. This research materialized with an article published in 2014.

Florin Petrariu, Ovidiu Alexinschi, Roxana Chirita, Vasile Chirita, Alin Ciobica, Manuela Padurariu, Radu Lefter, **Romeo Dobrin**, Radu Popescu, Emil Anton, Oana Arcan, Daniel Timofte, The dynamics of some oxidative stress markers in 3, 6 and 12-months alcohol abstinent patients: possible relevance for the usage of antioxidants in alcohol withdrawal, Revista Română de Medicină de Laborator , 2014, vol. 22, nr.4, pg. 451-457, **IF 2014 = 0.239**

**Scope**

In this way, for all the markers of the oxidative stress, as in the case of the main antioxidant enzymes SOD and GPX, there are previous reports stating both increased and decreased activities ((Ferreira, 2008)-increased; (Sutherland, 2013)–decreased, for superoxide dismutase) ((Montoya, 2013)-decreased; (Sies, 1997)-no modification at all, for glutathione peroxidase). Also, the other side of the oxidative stress balance, which is represented by the reactive oxygen species, is reported to suffer controversial modifications during the process of abstinence.Thus, when talking about MDA as the main marker of the lipid peroxidation processes, previous reports described either increased levels in patients with alcohol withdrawal (Hamad, 2013, Ciobica, 2011), as well as clear reductions in MDA levels following alcohol withdrawal (Montoya, 2013, Ciobica, 2011).

In our previous studies we showed a significant decrease of the oxidative stress status, one week and one month following the withdrawal, as demonstrated by a significant increase in the specific activity of SOD, as well as by a decrease in the MDA levels. However, as we showed back then, in the case of all three markers of oxidative stress status which we determined (SOD, GPX and MDA), the levels after one week or one month of abstinence were significantly altered when compared to controls (Ciobica, 2012).

Thus, in a continuation of our studies, in the present report we were interested in studying the importance of oxidative stress status in the alcohol withdrawal processes, by determining some oxidative stress markers after an even longer term: at 3, 6 and 12 months of abstinence and comparing them to the baseline and the control group.

**Materials and methods**

For this particular study, we used 62 patients selected between January 2013- July 2014, aged between 26 to 79 years old (average 44.8 ± 3.7 years), all of them males. The patients met the Diagnostic and Statistical Manual of Mental Disorders IV Text Revision diagnostic criteria for alcohol dependence. Of course, alcohol consumption was stopped abruptly at admission.

We excluded illicit patients, patients that used other drugs, patients with chronic systemic disease or severe mental disorders. All the patients were treated with benzodiazepines (diazepam or lorazepam).

In this way, we collected blood samples at baseline (N=33), at 3 months (N=14), at 6 months (N=14) and at 12 months(N=15), since some of them decided to drop out of the study or refused blood collection at some point.

The control group (N=32) included healthy, sex and aged-matched subjects without any psychiatric or physical illnesses. Also, the controls did not meet the criteria for alcohol abuse/dependence or any abusive alcohol consumption in the last 2 months.

Blood samples were obtained in the morning, before breakfast; after being centrifuged, the serum was then put into plastic tubes and stored at −40°C until measurement. Determination of SOD, GPX and MDA were performed by using "19160 SOD" or "GPX CGP1" Cellular Activity Assay Commercial Kits or by using classical and well known methods (for MDA - 10).

The current study was performed under the approval of the Socola Hospital Ethics Committee. Also, signed consent was obtained from all patients, according to the World Medical Association Declaration of Helsinki, revised in 2000, Edinburgh.

**Results**

After performing biochemical analyzes on the samples collected we observed significant changes in the activity of SOD enzyme between control group and study group ($p<0.0001$) (Figure 2.4), suggesting significant effects of alcohol abstinence on SOD specific activity.

Also, post hoc comparisons showed a significant increase in the specific activity in all 3 time-related abstinence cases, when compared to baseline results: ($p < 0.0001$ at 3 months), ($p < 0.0001$ at 6 months) and ($p < 0.0001$ at 12 months) (Figure 2.4). Still, there was a significant decrease in the SOD specific activity at 3 ($p <0.0001$) and 6 ($p < 0.0001$) months, when compared to the control group.

On the other hand, there were no significant modifications when we compared the control group with the 12 months group ($p =0.99$) (Figure 2.4). In addition, we also observed a progressive increase in SOD's specific activity, as the time from withdrawal increased, especially from 3 to 12 months ($p =0.0003$) and also from 6 to 12 months ($p =0.009$) (Figure 2.4).

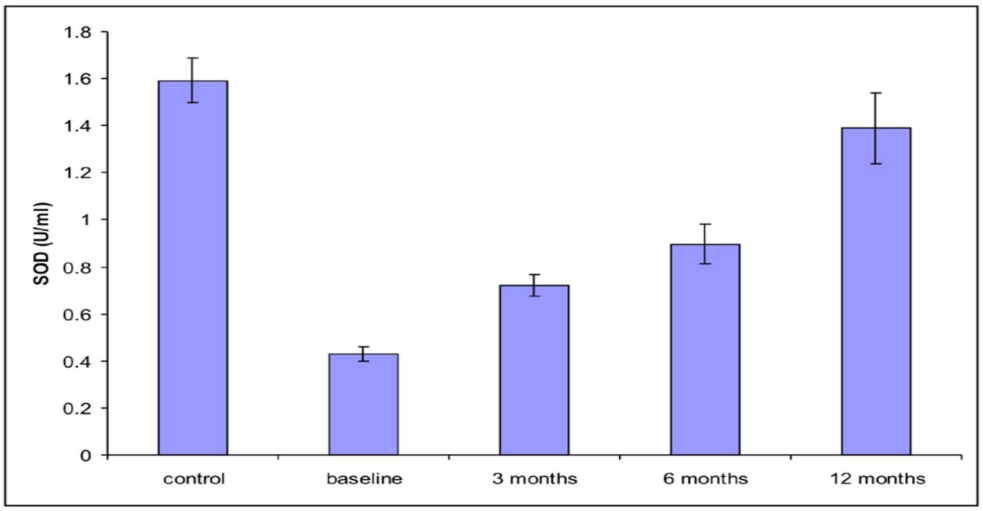


Figure 2.4. Superoxide dismutase (SOD) specific activity in the serum of control subjects, baseline and alcohol abstinent patients after 3, 6 and 12 months. The values are mean ± SEM (n = 32 in control group, n= 33 in baseline, n=14 in months group, n=14 in 6 months group and n=15 in 12 months group)

When it comes to the results of the other antioxidant enzyme, which was GPX, we could also observe a significant overall effect of the abstinence on enzymatic specific activity in our groups (p=0.0003) (Figure 2.5). Moreover, when we performed the post hoc analysis, we observed a significant increase in the specific activity of the enzyme, especially at 6 months (p =0.03) and 12 months (p =0.006), compared to the baseline group (Figure 2.5).

However, the specific activity at 3 months was still significantly decreased (p =0.026), when compared to the control group (Figure 2.5). Additionally, there was a progressive increase in the GPX specific activity from the time of withdrawal, as showed for example by the significant increase in the 6 months group, when compared to the 3 months group (p=0.007). Also, a significant difference was observed in the GPX specific activity between the 3 vs. 12 months group (p =0.001) (Figure 2.5).

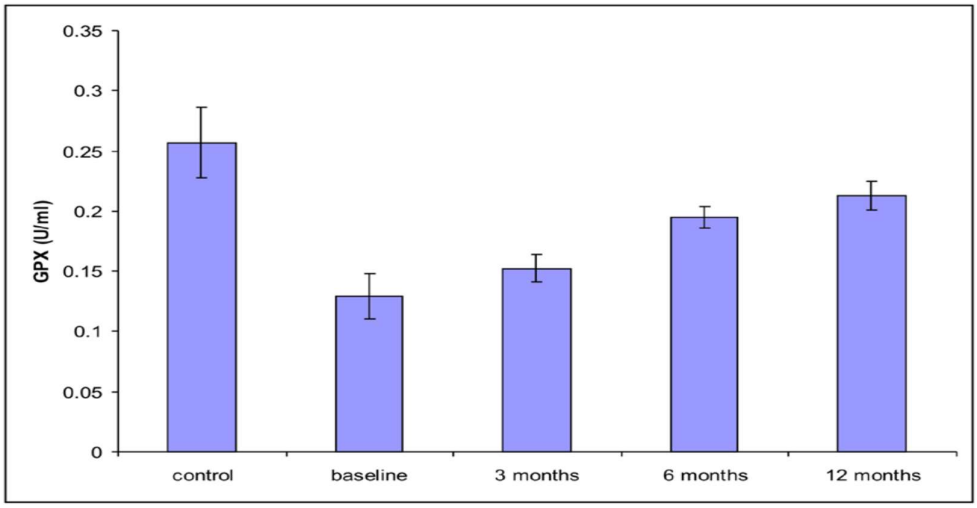


Figure 2.5. Glutathione peroxidase (GPX) specific activity in the serum of control subjects, baseline and alcohol abstinent patients after 3, 6 and 12 months. The values are mean ± SEM (n = 32 in control group, n= 33 in baseline, n=14 in 3 months group, n=14 in 6 months group and n=15 in 12 months group).

Regarding the levels of MDA, as a main marker for the lipid peroxidation processes, we also found significant differences between our study groups (p<0.0001). In addition to that, when we performed the post hoc analysis, we observed a significant decrease for all the 3 cases we studied, when compared to the baseline group (p =0.003 at 3 months), (p = 0.01 at 6 months) and (p = 0.0002 at 12 months) (Figure 2.6).

Still, no significant modifications were noticed when we compared our 3 study groups (p =0.07 at 3 months), (p = 0.19 at 6 months) and (p = 0.23 at 12 months) with the controls (Figure 2.6).

Also, we observed a tendency for a progressive decrease of MDA in time, as showed for example by the significant decrease of the MDA levels in the 12 months group, as compared to the 6 months patients (p < 0.0001). Furthermore, a significant difference was observed in the GPX specific activity between the 3 vs. 12 months group (p =0.0001) (Figure 2.6).

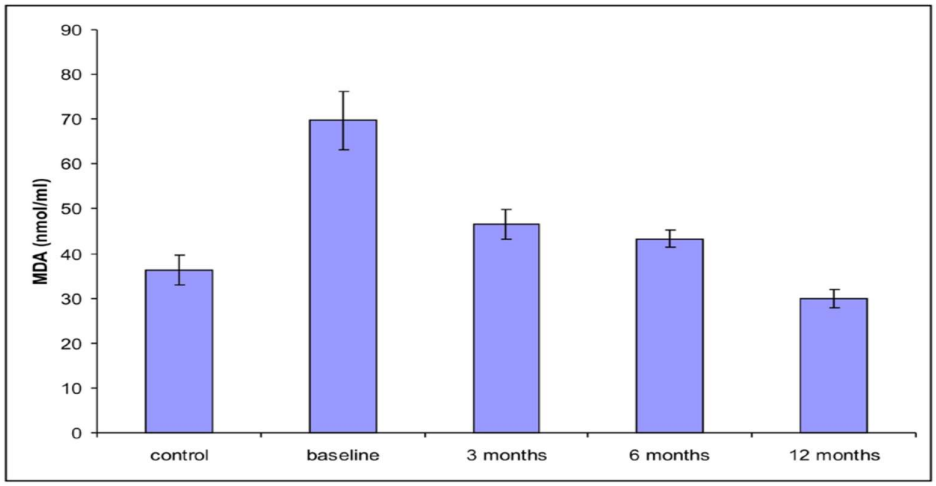


Figure 2.6. The levels of malondiadehyde (MDA) in the serum of control subjects, baseline and alcohol abstinent patients after 3, 6 and 12 months. The values are mean ± SEM (n = 32 in control group, n= 33 in baseline, n=14 in 3 months group, n=14 in 6 months group and n=15 in 12 months group).

**Discussion**

In this way, the data we presented in this study confirmed again the increased oxidative stress status in alcoholic patients and even more importantly, we showed that there is a significant and progressive decrease in the oxidative stress status at 3, 6 and 12 months after the withdrawal process, as demonstrated by the increased levels of antioxidant enzymes and decreased rate of lipid peroxidation, when compared to baseline values.

These data are also an important continuation of our previous studies, in which we demonstrated a decrease in the oxidative stress status, one week and one month following the withdrawal, as showed by a significant increase in the specific activity of SOD, as well as by a decrease in MDA levels, when compared to baseline. Still, in the case of all three markers of the oxidative stress status which we determined back then, the levels from one week or one month of abstinence were significantly altered when compared to controls, suggesting that severe and prolonged deficiency in their levels needs longer than one month of abstinence to normalize (Ciobica, 2012).

All these aspects could lead to the idea of using antioxidant compounds in order to reduce or improve the damages produced by alcohol consumption/withdrawal. In this way, it was showed for example that procysteine, which is a glutathione precursor (Padurariu, 2013), could increase the alcohol-depleted glutathione stores in various muscles of a rat model, following a period of abstinence, especially since it is known that alcohol consumption may result in numerous negative muscular effects (Lecomte, 1994).

Thus, glutathione restoration therapy could provide therapeutic benefits to the overall antioxidant state of skeletal muscles, especially when it is used in conjunction with an established detoxification program for the recovering alcoholics, as Otis et al. suggested (Otis, 2010).

Another important antioxidant drug in this area of research is represented by N-acetylcysteine, which was experimentally used, for example, for the myocardial oxidative stress in alcoholic heart disease Also, it seems that alcohol related oxidative stress could be in fact inhibited by N-acetylcysteine.

Moreover, there seems to be an interaction between N-acetylcysteine's metabolism and the withdrawal processes, which could result in decreased oxidative stress levels (Marotta, 1997). Importantly, as in the case of the procysteine, the protective effects of N-acetylcysteine could also be explained by the fact that it is required for glutathione biosynthesis (Huang, 2009a), which is of course an important antioxidant, with fundamental roles in preventing damages induced to important cellular components by the free radicals and various peroxides.

Thus, drugs like glutathione, procysteine and NAC are right now in our attention for their possible therapeutic actions in the withdrawal processes, both in animal models, as well as for human patient studies.

In this way, we generally demonstrated the fact that a decrease of the oxidative stress level is sustained by all measured parameters both on short term (Ciobica, 2012) and long term, as demonstrated through the results of the present report. Thus, the metabolism of the oxidative stress could be a fundamental aspect in the mechanistic of withdrawal and perhaps it may represent a central point where other negative factors are meeting, resulting in this complicated set of events.

However, there is a long way until we can establish a clear relationship between antioxidant- related deficiencies and alcohol consumption/ withdrawal time, especially considering the importance of free radicals in many metabolic reactions, but also due to the fact that in this reported study we actually showed a natural evolution for the oxidative stress status.

Regarding the limitations of the presented study, we could add the fact that all the groups received B vitamin supplements that could have influenced our results (however all the subjects received the same combination of B1+B6), also the lack of calculation power for this study, in order to see the number of subjects included (we rather used the patients which met the inclusion and exclusion criteria through the mentioned duration of the study, while trying to have a large enough group of subjects and controls), but also a more strict diet, body mass index and alcohol quantity determinations before withdrawal.

In summary, our results are suggesting that there is a significant and progressive decrease in the oxidative stress status at 3, 6 and 12 months after the withdrawal process, as demonstrated by the increased levels of antioxidant enzymes and decreased rate of lipid peroxidation, when compared to baseline values. This could be relevant for the beneficial and therapeutical actions of the antioxidants usage in the withdrawal processes.

Regarding the latest developments on the connection between oxidative stress and alcoholism, it was also proposed that oxidative stress may be an important mechanism associated with alcoholism especially in withdrawal state, but also with alcohol use or alcoholic liver injuries.

Regarding the involvement of oxidative stress in alcohol withdrawal, a recent study investigated oxidative stress parameters in 50 persons with withdrawal state from alcohol and compared with 50 matched. They reported a significant MDA level increase in alcohol withdrawal that remained increased even after the withdrawal state. These may suggest the oxidative stress may persist even after the clinical features of alcohol withdrawal had resolved Also, these authors reported a significant increase in the peripheral level of SOD level and a decrease of catalase concentration during withdrawal compared to the control group (Parthasarathy, 2015).

The mechanism by which alcohol induce brain damage in alcoholic individuals is not well understood. But a recent study investigated how oxidative stress affects the membrane of synaptosome in alcohol treated rats. Reddy and their collaborators treated the rats with 20% alcohol at 5g/kg body weight/ day and analysed the levels of some oxidative stress parameters. They found that in these alcohol-treated rats, the level of thiobarbituric acid reactive substances and protein carbonyls increased and the activity of catalase, glutathione peroxidase, superoxide dismutase decreased in synaptosomes. Also, alcohol administration decreased cholesterol/phospholipids ratio and increased membrane bound $Na^{+}/K^{+}$-ATPase, $Ca^{2+}$-ATPase and $Mg^{2+}$-ATPase enzyme activities suggesting impairment of membranal properties of synaptosomes due to oxidative stress. Moreover, they found that co-administration of vitamin E reversed these changes (Reddy, 2017).

Another review points out the connection between oxidative stress and alcohol that lead to brain damage, via ethanol oxidation in the brain. Practically, alcohol metabolism increases the production of the reactive oxygen species determining the oxidation of the fatty acids in phospholipids.

Consequently, the resulted bioactive aldehydes produced are known to be associated with may induce neurotoxicity and neurodegeneration (Hernández,). Moreover, the oxidative stress was demonstrated on a study on zebra fish exposed to alcohol. Agostini and his colleagues found that after chronic ethanol exposure, the brains of zebra fish showed an increase in TBARS and altered antioxidant activity reflected by altering CAT/SOD ratio was altered after chronic ethanol exposure, suggesting that alcohol exposure may induce oxidative damage in the zebrafish brain. Also, they found that alcohol inhibited choline acetyltransferase activity after acute exposure in 7 and 14 days but not after chronic exposure (28 days) (Agostini, 2018).

## 2.3 Biological and behavioral aspects regarding combined systemic management of alcohol dependence.

**A.Background**

Considering the complexity and the diversity of suffering caused by alcoholism related to the medical, familial, social and legal consequences of it, as well as the frequency of alcohol-related complex problems requires a more efficient biological approach (Alexinschi et al., 2014). It is well known that the consumption of alcohol is responsible for 2.3 million deaths per year worldwide, and in the United States alone more than 12 in 100 adults have met the criteria of DSM-IV for alcohol dependence at some point in their life (Friedman et al., 2013). Generally, there are two clinical entities that are associated with consumption in excess of alcohol: addiction and abuse (Abraham et al., 2011). Pharmacological treatment of alcoholism involves withdrawal treatment entailing detoxification, maintaining abstinence, but also the therapy of the medical complications (either somatic or psychiatric). The most commonly used drugs in alcoholism treatment are benzodiazepines, mood stabilizers, B-complex vitamins and pharmacological medication for maintaining abstinence. In this category belong antidipsotropic medications such as disulfiram, as well as medicines acting on opioid receptors and the GABAergic system (Cutler et al., 2005).

**B. Published article in this field**

The interest in this field materialized with the publishing of the following article.

Ovidiu Alexinschi, Roxana Chirita, Padurariu Manuela, Alin Ciobica, **Romeo Dobrin**, Daniel Timofte, Emil Anton, Raluca Prepelita, Carmen Anton, Vasile Chirita. Biological and behavioral aspects regarding combined systemic management of alcohol dependence. Archives of Biological Science, Belgrade, 67(1), 291-294, 2015, **IF 2014 =0,367**.

**Introduction**

Although there are numerous studies on the treatment of alcohol dependence, ranging from various schemes of pharmacologic treatment to specific psychotherapeutic approaches, the reviews and meta-analyses reveal only modest effects of these approaches, in many cases with the costs exceeding benefits. The crystallization and practical transposition of the ideology that consumption of alcohol is actually a type of behavior is represented by the Clubs of Alcoholics in Treatment (Hudolin et al., 1990; Mann et al., 1993). Further, we will present a prospective comparative study between associated management (behavioral systemic therapy plus standard therapy) versus the standard therapy alone of patients with alcohol addiction, as quantified by maintaining abstinence for 12 months and through the quality of life indicator's dynamics during treatment.

**Material and Methods**

In this study we involved 90 patients suffering from alcohol dependence that underwent clinical therapeutic evaluation within a period of 12 months. The patients evaluated in the study were in the files of the Clinical Psychiatric Hospital Socola in Iaşi. The patients were diagnosed with alcohol dependency in accordance with the criteria DSM-IV tr. These patients either presented or did not psychiatric or somatic comorbidity (with the exception of those specified in the exclusion criteria). All patients received standard medication for alcoholism (mood stabilizers, benzodiazepines and vitamin therapy).

Patients included in the study were aged between 18 and 65 years, with alcohol dependency (according to DSM-IV tr), who expressed their desire to stop drinking. We excluded patients who had completed treatment or psychotherapy for alcohol dependence during the past 90 days, patients without discernment, patients suffering from other associated somatic decompensated conditions which could worsen the patient clinical status during the participation in the study.

After screening, the patients were divided into two approximately equal groups (based on their consent to participate in the therapy carried out in the Clubs of Alcoholics in Treatment). Forty-four patients with alcohol dependence received standard medical treatment and entered a program of Hudolin behavioral systemic intervention and 46 patients received only standard medical treatment. The evaluation of patients with alcohol dependence was performed using the AUDIT scale (Alcohol Use Disorders Identification Test, Saunders et al., 1993) and the quality of life scales (QOL 16).

**Results and Discussion**

First the period of abstinence was evaluated. A significant ($F(1,66)=4.29$, $p=0.04$) difference of almost two months of abstinence between our study groups was recorded, since

the group which benefited from behavioral systemic plus standard therapy had an average of almost 7 months of abstinence (6.92±3.2 months), comparing to the group that received only standard medication (5.1±3.8 months) (as we see in Figure 2.7).

Afterwards, we performed the 6-month evolution for the quality of life scale scores in the two groups of patients as it can be seen in Figure 2.8. As is indicated in the figure, even if both groups of patients start with almost equal means values, after a month there is already a significant difference that persists during the subsequent months ($F(7,252)=21.25$, $p<0.01$).

These data are also supported by other studies showing the benefits of the systemic management. Some studies indicated an abstinence rate that goes up to 80% at 2 and 5 years associated with an increase in the quality of life in individual, marital, family, social and professional life (Gaöie et al., 1992).

Clinically we evaluate this important parameter, the quality of life index and improvement of this is another beneficial change and a progress indicator in the treatment of alcohol dependence. If the abstinence indicator is linked to consumption-associated behavior, the quality of life is more a marker of global change, which concerns not only giving up alcohol, but also beneficial changes in the personal life, family and social background of the individual. This indicator suggests a change in the patient's general behavior, a breakthrough, a personal development and spiritual growth and maturity. In assessing the effectiveness of systemic therapy, the use of this indicator of transformation is most appropriate, since this therapy aims precisely to improve the patient's personal life, growth and progress. In addition, these data were consistent with results obtained in the context of other observational studies (Alexinschi et al., 2006).

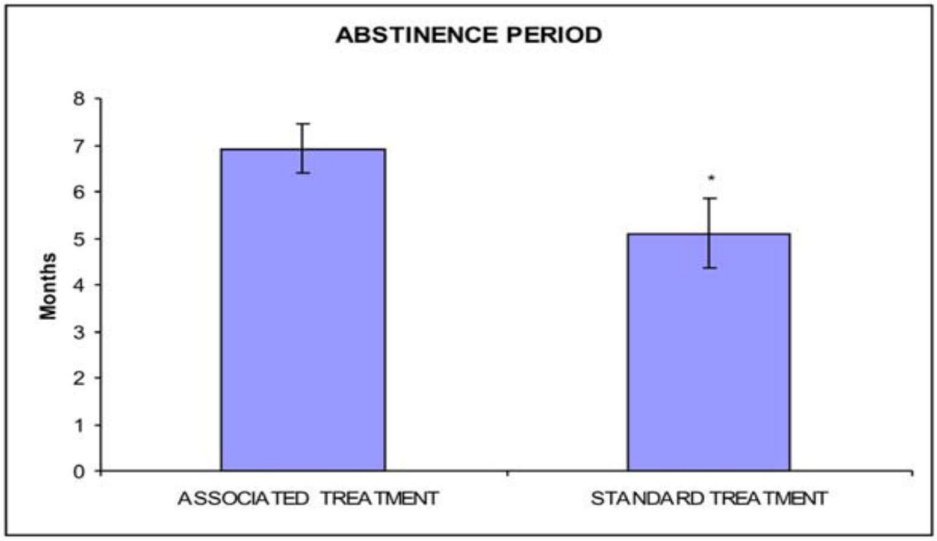


Figure 2.7. Comparison of abstinence period for the two groups of patients. The abstinence period for the two groups (behavioral systemic management plus standard therapy versus the standard therapy alone), as expressed in months. The values are mean ± SEM (n = 44 in the standard therapy group, n= 46 in the behavioral systemic management group). *p = 0.04. A comparison between the 6-month evolution of QOL16 scale for the two groups (behavioral systemic management plus standard therapy versus the standard therapy alone). The values are mean ± SEM (n = 44 in the standard therapy group, n= 46 in the behavioral systemic management group).

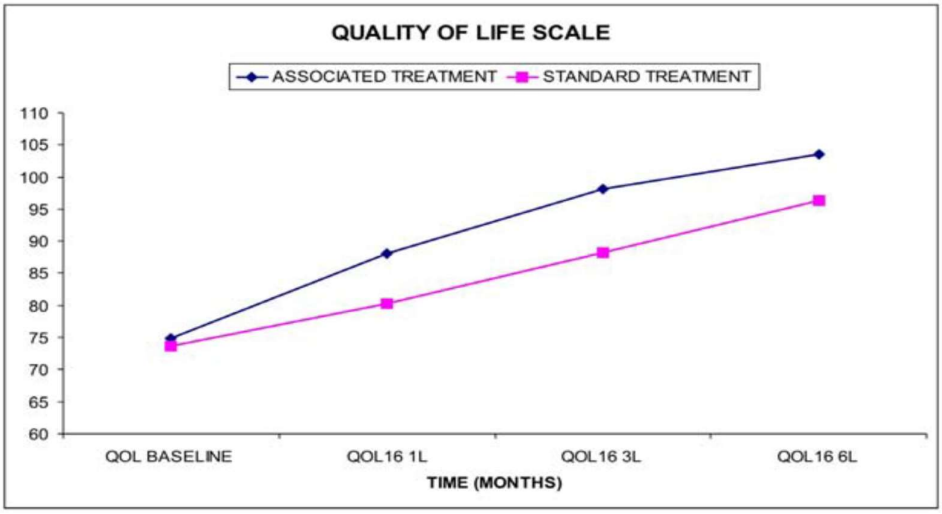


Figure 2.8**.** Comparison of evolution of QOL16-6 months between the two groups of patients. A comparison between the 6-month evolution of QOL16 scale for the two groups (behavioral systemic management plus standard therapy versus the standard therapy alone). The values are mean ± SEM (n = 44 in the standard therapy group, n= 46 in the behavioral systemic management group).

**Conclusion**

Considering that we observed an increased abstinence index, a better quality of life this clearly shows the benefits brought by systemic multi-behavioral and biological therapy in patients addicted to alcohol.

# SECTION I: CHAPTER 3:
# New insights regarding some biological mechanisms of the cognitive decline

**Introduction**

Cognitive decline is one of the main threats a psychiatris faces in current clinical practice. Although the main pathological changes are know for many of the etiological causes of cognitive decline, research in this field is of paramount importance as dementia stands to become the world's number one health issue.

In this chapter we syntethise the results of three studies. Two studies are fundamental research involving animal models and the physiopathology of Alzheimer's Disease and the third is a systematic review summarizing the main biomarkers, animal models, genetic perspectives, and antioxidant approaches in cognitive decline associated with affective disorders. Lately, the involvement of biometal dysregulation in some neuropsychiatric disorder has been on the spot line. In addition, increased interest regarding the biometal (BM) mechanisms of action and the pathways in which they have regulatory roles was lately observed. Particularly, it was shown that BM homeostasis dysregulation may lead to neurodegeneration including Alzheimer's disease, Parkinson disease, or prion protein disease, since important molecular signaling mechanisms in brain functions implicate both oxidative stress and redox active BM. Oxidative stress could be a result of a breakdown in metal homeostasis which leads to abnormal metal protein chelation. What we found was an increased lipid peroxidation effect, low antioxidant defense, low magnesium and iron concentrations, and high manganese levels in MCI and AD patients. Previous research suggested some contradictory results concerning the BM serum levels in neurodegenerative disorders. Recent studies reported various tendencies in BM level dynamics in demented patients (Gao, 2008, Farina, 2013).

The growing interest on OT is also based on the demonstrated beneficial effects as a stress reliever and a social bonding agent. The second study looks into behavioral changes induced by short-term intraperitoneal oxytocin administration in aged rats. Our results provide evidence regarding the memory, mood and social behavior changes induced by intraperitoneal OT administration. The fact that peripheral OT administration leads to observable changes at central levels suggests that intraperitoneal route of administration may also be as effective as the intranasal route.

## 3.1 Preliminary Data on the Interaction between Some Biometals and Oxidative Stress Status in Mild Cognitive Impairment and Alzheimer's Disease Patients

### A. Background

Lately, the involvement of biometal dysregulation in some neuropsychiatric disorder has been on the spot line. Also, increased interest regarding the biometal (BM) mechanisms of action and the pathways in which they have regulatory roles was lately observed. Particularly, it was shown that BM homeostasis dysregulation may lead to neurodegeneration including

Alzheimer's disease, Parkinson disease, or prion protein disease, since important molecular signaling mechanisms in brain functions implicate both oxidative stress and redox active BM. Oxidative stress could be a result of a breakdown in metal homeostasis which leads to abnormal metal protein chelation.

It is now generally accepted that several BM such as iron, copper, zinc, manganese, and magnesium are vital in the complex cellular activities and regulation. [1–5]. Lately, research focuses on BM mechanisms of action due to their capacity to lead to several pathway degeneration when homeostatically impaired. In this way, neurodegenerative diseases such as Alzheimer's disease, Parkinson disease, and prion protein disease are shown to be closely related to several BM levels.

Regarding Alzheimer's disease (AD), it is important to say that it is a complex progressive disorder involving both behavioral and molecular distress. In this way, it is now accepted that AD is a multifactorial disease in which several important components have been described: the discrete biochemical changes which firstly occur triggering cellular modifications such as amyloid accumulation and neurofibrillary tangles formation; the histological typical features accompanied by synaptic disruption and neuronal loss; and last but not the least the visible symptoms of the cognitive and behavioral component—memory loss, attention deficit, disorientation, judgment impairment, restlessness and related comorbidities (affective distress and somatic disorders such as chronic pain or anemia) (Ikbal, 2010, Alkadhi 2011, Carreiras , 2013, Morris, 2014 –9).

Another important clinical entity that is associated with cognitive decline is mild cognitive impairment (MCI) which is a disorder providing a major risk factor for AD [10]. Moreover, while some overlapping traits between MCI and the early stages of AD in considering the characteristic mild cognitive decline were suggested, it was shown that AD cognitive abilities gradually decline, but MCI patients' cognitive state remains stable for years (Boyle, 2006).

Oxidative stress is also an important pathway of AD pathology. The common knowledge on aging now includes a biochemical theory that partly explains the cellular decline due to oxidative/antioxidant process imbalance occurrence at a cellular level as we age. Together with that, the thorough description of brain biochemical mechanisms which revealed its high oxygen resources needs and its special membrane lipid-rich structure leads to the conclusion that brain tissue is extremely susceptible to oxidative stress. In this context, it was demonstrated that oxidative stress plays important roles in AD pathology, both at its first molecular changes and also during its development up to its final stages.

Oxidative stress may be the common ground in metal-ion homeostasis which leads to abnormal metal protein chelation. Extensive evidence points to an important implication of several both toxic and redox metal ions which can contribute to DNA and protein damage causing oxidative stress and molecular damage (as reviewed by Jomova, 2011) by being involved in cycles of electron transfer reactions from and to the substrates which make them extremely important in redox and metal homeostasis, both of which are tightly related (Lindeque, 2010).

Therefore, any ionic metal unbalance occurring at the cellular or peripheral level is reflected in abnormal redox homeostasis followed by excessive reactive oxygen species (ROS) production, oxidative stress, and their further effects (Valko, 2007).

**B. Published article in this field**

Our inquire into the role biometals play in Alzheimer's Disease pathophysiology was materialized with the publication of the following article:

Ioana-Miruna Balmuș, Stefan-Adrian Strungaru, Alin Ciobica, Mircea-Nicusor Nicoara, **Romeo Dobrin**, Gabriel Plavan, Cristinel Ștefănescu : Preliminary Data on the Interaction between Some Biometals and Oxidative Stress Status in Mild Cognitive Impairment and Alzheimer's Disease Patients, Oxidative Medicine and Cellular Longevity, Volume 2017, Article ID 7156928, 7 pages , **IF 2017 = 4,93**

**Introduction**

The critical role of copper, iron, and other trace redox-active transition metals was shown recently to be implicated in the pathogenesis of AD [14, 15]. While our group previously suggested a strong link between oxidative stress and Alzheimer's disease (Padurariu, 2010, Padurariu, 2013) we aimed to assess the possible cause/effect relationship between BM abnormal levels dynamics and the increased oxidative damage occurring in AD pathology. Moreover, we demonstrated a progressive pattern of oxidative markers change during both mild cognitive impairment and Alzheimer's disease patient's analysis which could also be linked to a progressive BM level pathological tendency.

Considering all the information aforementioned, the aim of the present work was to evaluate some relevant BM levels (magnesium, manganese, and iron), the specific activity of some antioxidant enzymes such as SOD and GPX and MDA levels as a marker of lipid peroxidation, in MCI and AD patients, compared with age-matched control group.

**Material and Methods**

Regarding the material and method used for this study, we recruited a number of 30 patients with cognitive decline, among which 30 patients were diagnosed with AD and 15 patients with MCI. The control group was consisted from healthy age- and sex-matched participant (n = 15). Blood samples were collected from all the patients included in the presented study. All the participants were recruited from the "Socola" Regional Institute of Psychiatry (Iasi, Romania) based on ethical agreement from the Regional Institute of Psychiatry Board Committee.

Also, the cognitive status of the participants was assessed using standard Mini-Mental State Examination (MMSE) (Mohs, 1983) and Alzheimer's Disease Assessment Scale-Cognitive (ADAS-Cog) [19]. All the AD patients underwent psychiatric diagnosis fulfilling NINCDSADRDA criteria and ICD-10 criteria (McKhan, 1984, Kelley, 2008). Also, MCI diagnosis followed Petersen et al. criteria (memory impairment accompanied by general cognitive and functional abilities preservation) (Petersen, 1999).

The study was conducted according to Helsinki Declaration and national and European regulations on Biomedical Research. All the patients or their families were given and signed a written informed consent for their contribution in this study. Several exclusion criteria were provided for patients' recruitment such as antioxidant supplementation and acute or severe comorbidities.

Further one, after blood samples were collected (a jeun) they were allowed to clot. After centrifugation (3000 rpm, 15 minutes, 4°C), blood sera were separated, aliquoted, and stored at

−22°C until analysis. For BM quantification, the stored aliquots were processed according to the following protocol: 1 ml of sample was digested with 3 ml nitric acid 65% and 2 ml of hydrogen peroxide in decontaminated TFM pressure vessels that were inserted in Speedwave MWS-2 produced by Berghof. The digestion program for samples was in steps as follows: 145°C for 5 min, 190°C for 10 min, and 100°C for 10 min [23]. After the microwave digestion, the samples were transferred in 25 ml decontaminated flasks and filled up to volume with ultrapure water. No special preparation except for the biochemical analysis kit brochures' mentions were needed for the biochemical analysis protocols.

In order to quantify the biometals, high-purity and producer-certified-quality reagents were used for the element separation and measurement. Ultrapure water filtered by LaboStar™3/7 TWF (Siemens) purification system from double-distilled water was used for decontamination, sample preparation, and reagent dilution. High-purity nitric acid 65% (Merck, Germany) and hydrogen peroxide EMSURE 30% stabilized for higher storage temperature (Merck, Germany) were used in the metal digestion process from the biological samples. The standard stock solutions for AAS used in the calibration method were certified by Merck, Germany. All the necessary solution used in calibration and quantification of the metals were manganese (1000 mg/l), iron (1000 mg/l), and magnesium (1000 mg/l).

Atomic absorption spectrometer with a high-resolution continuum source equipped with graphite furnace and platform, ContrAA 600 from Analitik Jena, Germany, was used for all the element measurements. Matrix sample modifiers diluted from certified solutions of $Pd/Mg(NO_3)_2$ (Merck Germany) were necessary. Blind samples for testing any possible contamination from laboratory and reagents were prepared. Standard solutions were used for quality-control sample preparation consisting in various successive concentrations.

**Results**

The biological measurements of the stress oxidative status, including SOD, GPX enzymes together with the MDA, the product of lipid peroxidation was described before in the present report (please see the first and the second chapter regarding the implications of oxidative stress in schizophrenia and alcohol withdrawal).

In this particular study, we measured several important BM levels, in the peripheral blood of MCI and AD patients, compared with age- and sex-matched controls. Chemical analysis of the subjects' blood serum revealed significant differences regarding the magnesium, manganese, and iron levels in the MCI and AD patients' serum.

We observed a decrease in the iron level in AD patientsn(140.43 ± 16.02 μg/dl) as compared with the healthy sex- and age-matched controls (192.45 ± 42.51 μg/dl). Also, interestingly, we firstly observed a slight increase of the iron levels in the MCI patients (242.47 ± 18.06 μg/dl) followed by the significant decrease in AD patients (Figure 16). Overall, one-way ANOVA showed a significant variation between the groups: C – MCI−AD [$F(2, 30) = 3.82$; $p = 0\ 033$]. Post hoc comparisons using the Tukey test indicated that the mean score for the AD group ($M = 140\ 43$, $SD = 57.77$) was significantly different than the MCI group ($M = 242\ 47$; $SD = 57.12$).

Regarding the manganese levels, we observed a statistically significant increase of manganese levels in AD patients (3.32 ± 0.07 μg/dl, $p < 0\ 001$) and MCI patients (3.10 ± 0.09 μg/dl, $p < 0\ 05$) as compared to the healthy sex and age-matched controls (2.68 ± 0.16 μg/dl)

(overall ANOVA: F(2, 32) = 8.93, p = 0 0008) (Figure 3.1). Post hoc analysis revealed that the mean score for the MCI and AD groups were significantly different from the control group regarding the manganese levels and similarly regarding the magnesium levels.

On the other hand, we observed that AD patients' (994.08 ±69.04 µg/dl, p < 0 01) and MCI patients' (1051.40 ± 65.43 µg/ dl, p < 0 05) magnesium levels tend to decrease as compared to those of healthy controls (1316.46 ± 60.27 µg/dl) (Figure 3.1). Also, overall one-way ANOVA analysis revealed a significant pattern of magnesium level decrease [F(2, 35)= 4.73; p = 0 015] for the experiment groups, as compared with the healthy sex- and age- matched controls.

Regarding the BM levels trending, several direct moderate and low correlations (post hoc Pearson's correlation) have been observed. In this way, while analyzing iron against manganese trending, a moderate negative correlation has been observed (Fe-Mn: r = −0 431, p = 0 012). Weak negative correlation was obtained while analyzing magnesium versus manganese trending (Mg-Mn: r = −0 27, p = 0 12), and no statistical correlation was found for iron versus magnesium comparison.

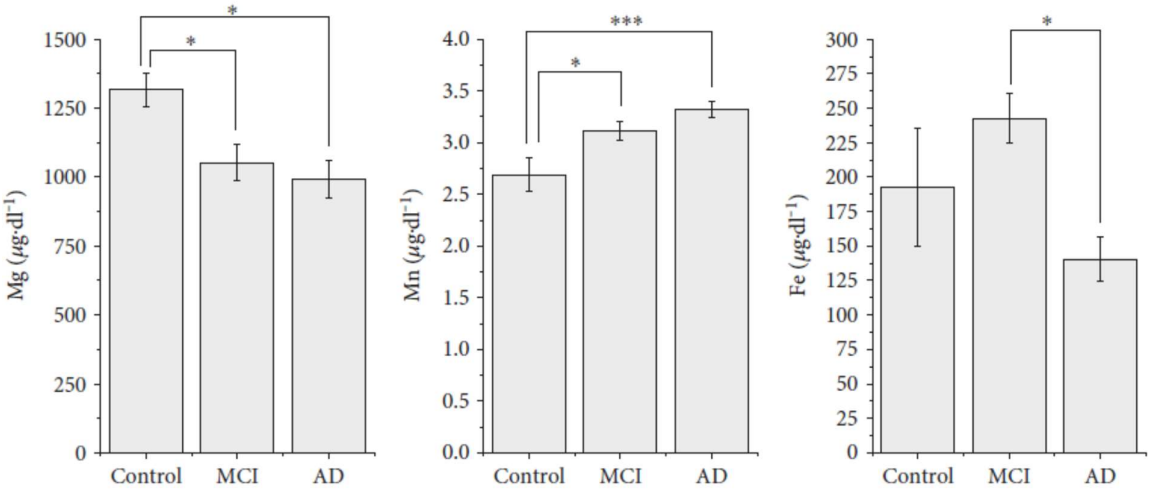


**Figure 3.1:** Magnesium, manganese, and iron concentrations in blood sera (presented as mean ± SEM) in studied groups (∗$p$ < 0 05 and ∗∗∗$p$ < 0 001, Tukey HSD test).

However, while comparing BM levels with specific psychiatric scales applied to the patients, we observe a strong negative correlation between MMSE score and manganese levels (r = −0 585, p < 0 001) (Figure 3.2).

Interesting results were obtained in post hoc analysis (Pearson correlation) of biometal concentrations versus oxidative stress status correlations. In this way, we obtained significant statistical correlations for two of the oxidative stress markers as compared with manganese concentrations (Mn-GPx: r = −0 564, p < 0 001; Mn MDA: r = 0 561, p < 0 001). For magnesium levels, moderate statistical correlations were obtained for Mg-GPx (r = 0 509 p = 0 002) and Mg-MDA (r = −0 383, p < 0 05) comparisons. No statistical significant correlations were found during post hoc analysis of serum iron levels and oxidative stress markers.

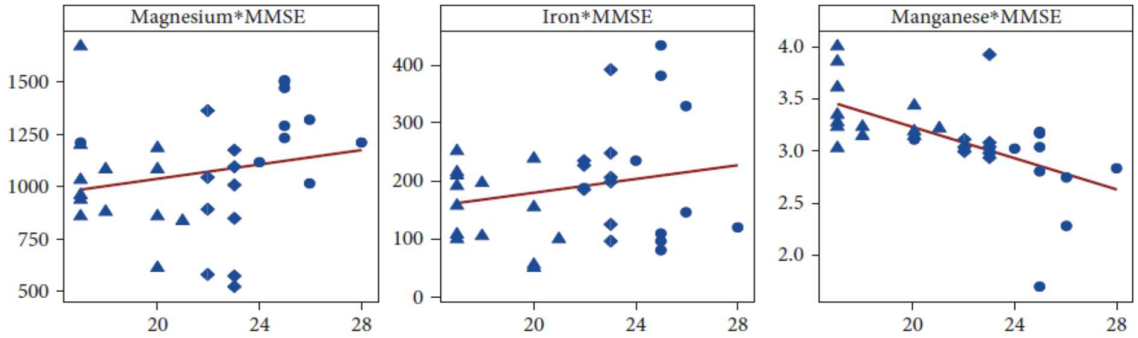


**Figure 3.2:** Correlation between MMSE scores and BM levels in control (●), MCI (♦), and AD (▲) patients (explanation in text).

**Discussions**

Furthermore, we will discuss the obtained results comparing with the results observed by other asuthors regarding this subject. The present work aimed to evaluate manganese, magnesium and iron levels, the specific activity of some antioxidant enzymes (SOD and GPx), and MDA levels as a marker of lipid peroxidation therefore cellular damage, in MCI and AD patients, compared to age-matched healthy subjects.

What we found was an increased lipid peroxidation effect, low antioxidant defense, low magnesium and iron concentrations, and high manganese levels in MCI and AD patients. Previous research suggested some contradictory results concerning the BM serum levels in neurodegenerative disorders. Recent studies reported various tendencies in BM level dynamics in demented patients (Gao, 2008, Farina, 2013).

In this way, Barbagallo et al. (Barbagallo, 2011) showed decreased magnesium ion levels in AD patients' serum. Also, Cilliler et al. (Cilliler, 2007) reported a correlation between low AD group magnesium levels and MMSE score. Similarly, manganese level analysis in the recent studies revealed controverted results.

The most recent meta-analysis on the matter, Du et al. (Du, 2017) reports controversial dynamics of manganese levels presenting several studies which show a significant decrease of this parameter in demented patients while in other studies, no difference or significant increase were obtained. We also found statistical correlations between MMSE scores versus manganese levels and ADAS-COG versus magnesium and manganese levels.

Regarding the iron levels, the previous studies suggest no significant change in demented patients (Wang, 2015) while the more recent Paglia et al. (Paglia, 2016) study reported a progressive decrease of this parameter in AD, MCI patients, subjective memory complaint, and healthy participants.

In our study we report similar tendencies of the discussed BM in MCI and AD patients, as compared to age- and sex-matched control group and also significant statistical correlations for all the studied biometals versus lipid peroxidation marker, moderate statistical correlations for antioxidant enzymes versus magnesium levels. Also, there wasno statistical significant correlation between oxidative stress markers and iron levels. However, an interesting iron level pattern of progression was observed. Previous studies show high specific antioxidant enzymes activities (Aybek, 2007, Baldeiras, 2008) and a compensatory activity in the body. Also, several studies reported decreased nonenzymatic antioxidant factor levels and high iron, aluminum, and mercury concentrations in demented patients (Webster, 2001). Furthermore, clinical trials

showed an increased oxidative stress markers' levels in patients with MCI as well (Torres, 2011, Cervellati, 2014, Swomley, 2015).

Also, similar iron pattern of variation was reported by Paglia et al. (Paglia, 2016) who showed that the iron level increase (in individuals showing subjective memory complaint) is followed by abrupt concentration decrease in MCI and AD patients. These findings, together with the study of Smith et al. (Smith, 2010) which show increased iron levels in AD and MCI brains forming iron deposits could suggest that anemia may be a common condition found in AD patients.

This idea was also suggested by Hare et al. (Hare, 2015) who reported low iron plasma levels in correlation with low-bound transferrin levels. Moreover, our findings could be explained by the importance of iron in oxidative stress modulation. While high levels of iron were observed in demented patients' brain and high oxidative stress is a common trait for AD, the low-iron serum levels could suggest that intense brain redox activity is resulting in excess iron concentrations in that tissue. This may be the reason why an increased iron-modulated immune response could be observed in the brain tissue during initial accumulation of beta-amyloid plaques (Martorell, 2017).

However, while our previous results show increased serum oxidative stress levels, it seems that this variation is not dependent on iron activity. Although controversial results were obtained in similar studies which showed that manganese levels in demented patients are decreased, many reports demonstrate that manganese could contribute to beta-amyloid plaque formation (Tong, 2014) and therefore an increase of this parameter would be possible both in brain tissue and also in blood serum. In this way, Tong et al. reported a plasma Aβ peptides concentration increase in association with high serum manganese levels.

Despite the fact that manganese was shown to significantly increase hippocampal glutathione peroxidase (GPx) activity (Peres, 2015) and manganese dependent SOD (SOD2) [45, 46], it seems that an inverse correlation may be observed in peripheral blood of demented patients regarding GPX activity and the enzymatic activity of SOD. However, Dobson and Aschner (Dobson, 2007) extensively discuss the oxidative stress induction potential of excess manganese. Many redox activity molecular studies on effects of manganese accumulation led to mitochondrial oxidative stress pathway description. It seems that excessive manganese could lead mitochondrial electron transport chain perturbance, which eventually causes additional ROS, cellular oxidative stress, and furtherly apoptosis.

Also, in the redox reactivity series of metals, manganese occupies a leading position; therefore, it possesses the highest potential to generate ROS. In this way, excessive manganese intake may lead to mitochondrial dysfunction and oxidative stress alongside neuronal and astrocytic apoptosis. This aspect could be observed in the strong positive correlation between manganese and MDA levels which reflects the effects of excessive ROS production through molecular level causing lipid peroxidation and cellular damage.

The role of magnesium deficiency in AD pathogenesis has been extensively discussed (Glick, 1990). Moreover, Durlach (Durlach, 1990) brings several more arguments on magnesium mechanisms of modulation in glutamatergic transmission and hippocampal activity. Also, in an AD mouse model, Li et al. (Li, 2014) points to imminent recovery of cognitive deficits and synaptic loss following magnesium administration. Xu et al. (Xu, 2014) reported that magnesium deficiency in rats may lead to increased free radical oxygen species,

also introducing the magnesium implication in inflammation and oxidative lesions. Moreover, the correlation between magnesium and the two antioxidant enzymes (GPx and SOD) is entitled since the high rate of ROS production in the presence of low antioxidant activity could be a result of magnesium depletion.

In summary, our data show progressive increase of manganese levels in demented patients, as compared with healthy controls and progressive decrease of magnesium levels in the groups. A slight increase in iron serum levels was observed in MCI patients followed by an abrupt level drop in AD patients. We observed several strong correlations between cognitive status and biometals serum levels.

Also, several correlations between the studied biometal serum levels and main oxidative stress markers were observed. Positive correlations were found in manganese versus MDA, magnesium versus GPX, and magnesium versus SOD analysis and negative correlations in manganese versus SOD and manganese versus GPX analysis and no correlations were found between serum iron levels and serum oxidative stress markers. These data could be relevant for future studies on the prediction of AD development risk and progression pattern by analyzing the relation between active redox metals and oxidative stress markers.

Regarding the latest developments (after the publication of our aforementioned article) on the correlation between AD and the neuropsychiatric disorders in general and most of the biometals and elements, in the context of the oxidative stress relevance, we can mention for example the study of Cavado et al which showed that biometals could act as potential predictors of the neurodegenerative decline in Alzheimer's disease (Lavado et al. 2019). Thus, the Cavado group focused their attention on cooper, zinc, magnesium, calcium, iron and selenium (e.g. for example it is known that iron is associated with the neuroinflammatory processes in the AD pathology, while calcium, cooper and zinc could mediate the pathological processes of dementia) (Lavado et al. 2019).

Moreover, all these metals could be related to the excess of protein deposits in the brain, which are an important hallmark for these dementia-like disorders, such as the deposition of Amyloid-β (Aβ) peptide, Metallothionein 3 (MT3), Tau protein, Amyloid-β Protein Precursor (AβPP) or presilins etc (e.g. as the authors are describing it is possible that "magnesium and calcium interfere with the cleavage of AβPP and presenilin function, which further causes the formation of Aβ aggregates and oxidative stress.") (Lavado et al. 2019).

Similar aspects were also demonstrated in 2017 in a publication in Frontiers in Molecular Neuroscience by the Yong team (Li et al. 2017), focusing for example on most of the biometals and their transporters, in the context of the dementia-general- related pathology in the case of iron, cooper, zinc, manganese, magnesium and calcium, as well as very interesting perspective on the non-biologically relevant metals and its toxicity (Li et al. 2017).

Even more, in 2019 it was demonstrated in a conclusive article by Strugaru et al. that there are important correlations between the acute exposure to methylmercury chloride induces fast changes inswimming performance, cognitive processes and oxidative stress of zebrafish (Danio rerio) as a reference model in the present context (Strungaru et al. 2018)

## 3.2: Preliminary data on some behavioral changes induced by short-term intraperitoneal oxytocin administration in aged rats

### A. Background

Oxytocin (OT) is a well-known neuropeptide produced mainly in the hypothalamus in the magnocellular neurons from the supraoptic and paraventricular nuclei and together with other peptides such as vasopressin, melatonin, insulin and other hormones can alter both behavior and physiology of neuronal functions (Lischke et al. 2012).

The growing interest on OT is also based on the demonstrated beneficial effects as a stress reliever and a social bonding agent. Several studies actually review these psychosocial roles of OT (Meyer-Lindenberg et al. 2011, Striepens et al. 2011) and conclude that these findings encourage the potential therapeutical effects of OT in several psychiatric disorders, such as the affective disorders and the autistic spectrum disorders.

One important aspect is that neuropeptides such as OT may have a modulatory effect on the brain altering several limbic regions activity patterns. In this way, OT may induce both amygdala activation and inactivation when exposed to adverse social stimuli (Lischke et al. 2012). This is the reason why, it was concluded that the OT roles very much depend on the social and emotional context of the individual when OT is administrated. In this context, the nature of the stimuli, the gender and the genotype, have been demonstrated to be influencing the OT pattern of action (Striepens et al. 2011).

### B. Published article in this field

Our interest in this field materialized with the publishing of the following article:

Ioana Miruna Balmus, Radu Lefter, Alin Ciobica, Iulia Antioch, Daniela Ababei, **Romeo Dobrin**, Preliminary data on some behavioral changes induced byges induced by short-term intraperitoneal oxytocin administration in aged rats**,** Psychiatria Danubina, Vol.30, No.1, , March 2018, pg. 91-98, **IF 2018 = 0,683**

#### Introduction

Several studies also show difference in OT activity and receptor distribution and ways of transportation. Thus, it has been shown that the administration method can crucially influence the OT penetration of blood-brain barrier. In this way, many studies showed significant cerebrospinal fluid OT levels after intranasal administration both in humans (Striepens et al. 2013) and rats (Veening et al. 2010), but also in macaques (Dal Monte et al. 2014). By contrast, animal-based studies showed that less than 0.1% of the administered OT can cross the blood-brain barrier by intravenous mean (Kendrick et al., 1991).

Moreover, very brief half-life of OT is noted especially when administered peripherally (Mens et al.1983) and more recently it has been proven that in fact oxytocin penetrate the hemato-encephalic barrier. In spite that, there are several studies that suggest several correlations between intraperitoneal administration of OT and social affiliation increase (Mooney et al. 2014), low anxiety relief (Crine et al. 1983), but strong effects on nerve recovery following nerve damage (Gümüs et al. 2015).

Regarding the relevance of oxytocin in aging and cognitive deficits, several studies show a relation between OT plasma levels and cognition and behavior deficit (Lancaster et al. 2015, Valstad et al. 2016, Zhong et al. 2012). The association between old age and OT was only vaguely studied. Little or few is known on the effect of the OT hormone on the old body. For instance, OT beneficial effect has been demonstrated in old age context regarding the reparatory yield improvement of old muscles exposed to OT. In this way, Elabd et al. (2014) shows that plasma OT and receptors' levels of OT in muscle dramatically decline during aging and demonstrate that muscle tissue regeneration and homeostatic maintenance is dependent on the OT levels.

Actually, a similar mechanism was suggested regarding the effect of OT in the brain. In this way, Parker et al. (2010) show that CSF OT levels in female Rhesus monkeys may be associated with central nervous system alterations due to old age and, moreover, may be used as a lifespan developmental biomarker for age-related central nervous system changes. In this context, we aimed to examine the OT effects on several cognitive functions in old age individuals. Further one, we, will present some of our preliminary research regarding the intraperitoneal administration of OT for 8 days in some specific behavioral tasks assessment for anxious and depressive behavior and sociability) with Y-Maze test for short-term working memory, Open Field test, Elevated Plus Maze, and Forced Swim test for and Three-chamber Maze.

**Materials and Methods**

Methodologically speaking, in order to realize this objectives, we used two groups of male Wistar rats were used for this study, divided based on their age: control group (n=5) consisted in aged male rats (two years old) which received intraperitoneal injection with saline solution (Aged + Saline), and treatment group (n=5) consisted in aged male rats which received intraperitoneal injection with oxytocin solution (Aged + i.p. OT). The animals were housed in polyacrylic cages (5 animals/cage) containing woodchip bedding, and maintained in a temperature and environment-controlled room (22±2°C, a 12-h cycle, relative humidity of 40-60%). The animals were acclimatized to laboratory condition for 10 days before the beginning of the experiment. Rats were fed a standard laboratory diet of rat chow pellets, according to the McCollum diet requirements. Except for the periods of the behavioral tests which require food limitation and two hours before OT administration, food and water were available ad libitum.

Rats were treated in accordance with the current national and European Regulations regarding the scientific research using animals and in accordance with NIH- Care and Use of Laboratory Animals Manual (8th Edition) and in accordance with the guidelines of animal bioethics from the Act on Animal Experimentation and Animal Health and Welfare Act from Romania and all procedures were in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Rats were intraperitoneal injected with OT (Sigma-Aldrich Co. LLC., Darmstadt, Germany) in a dose of 10 mg/kg/body weight for 8 days consecutively. Control animals received intraperitoneal administration of the same volume of OT vehicle. The treatment began 6 days before the behavioral testing. After that, rats were subjected to a battery of behavioral tests in the following order: Y maze test, elevated-plus maze, open field test, forced swim test,

splash test, and Trichamber sociability test performed from the last 2 days of treatment to the second day past the final treatment day.

Y-maze test used is a reliable, non-invasive short-time memory test, used to determine the cognitive changes in Wistar rats occurred in the spontaneous alternation task as a behavioral marker for the short term memory function. The maze used in the present study consisted of three arms (35 cm long, 25 cm high and 10 cm wide) and an equilateral triangular central area. The animals were tested following the standard protocol described by Kokkinidis et al. (1976).

Another test used for assessing anxiety-like behavior and locomotor activity was Open Field test (OFT). An open field rectangular apparatus consisting in an empty open area with white floor (plastic-covered flooring) and white walls was placed in a low illuminated room, far from the room walls and furniture. The animals were observed for 5 minutes and evaluated.

Elevated Plus Maze test analyzes anxiety-like behavior and is consisted in two open arms black Plexiglas arms, and two enclosed black Plexiglas arms with black lateral walls and open roof, opposite to each other and elevated from the ground at the height of 50 centimeters, was placed in an illuminated room, far from the room walls and furniture. The behavior markers were assessed following the standard protocol methodology described by Pellow et al. (1985).

Forced Swim test (FST) assesses behavioral despair as a marker for depressive behavior The test apparatus consisted in a transparent glass cylinder containing water (23–25°C, 30 cm depth). The animals were placed in the water for 15 minutes. The next day, the rats were re-exposed to the swim arena for a 5-minute period. Using time-sampling method, the animal behaviors were assessed as following: immobility time (floating), swimming time (horizontal movements) and climbing (vigorous upward movements) (Detke et al. 1995). The splash test (ST) was performed by spraying a 10% sucrose solution on the dorsal part of the rats in their home cage which induces grooming behavior. The latency to the first groom, the number of grooms, and total duration of the grooming were recorded for 5 minutes (Zou et al. 2015). Depressive behavior is characterized by increased latency time, and decreased time spent grooming.

In order to assess sociability and social behavior expressing species such as mice and rats, Crawley et al. (2004) suggests using a device consisting in three chambers (central compartment, and lateral compartments, so that it is given the choice of whether to interact or not with an unknown animal), three-chamber sociability test (TCT).

The 30 minutes test consists in three 10 minutes phases: the first phase in which the evaluated animal is placed in the central compartment and allowed to explore the apparatus; in the next phase, a new animal is placed in one of the lateral chambers; and in the last phase, another one is added to the other lateral chamber. The time spent exploring the chambers, the phase I animal, the number and duration of contacts, the preference for the phase II animal were evaluated. Sociability indicates the preference for the novel mouse compared to the empty compartment or known animal.

**Results**

When we analyzed the behavioral parameters obtained in YMT, we obtained a mild increase in spontaneous alteration in OT group (55.82±2.68%), as compared to control group (49.33±4.94%) (Figure 3.3), that may suggest a mild influence on short-term spatial memory ($F(1,6)=1.32$; $p=0.29$).

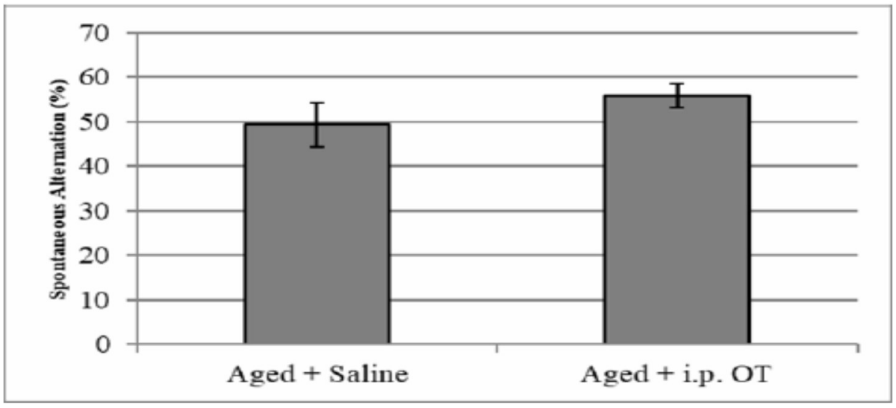


Figure 3.3. The effects of intraperitoneal OT administration in the Y-maze test, as showed by the spontaneous alternation behavior. The values are expressed as mean ± S.E.M. (n=5, per group).

When we analyzed the rat behavior in the FST, we evaluated several parameters such as general mobility, struggling duration, and floating duration. In this way, regarding the general mobility, we obtained considerably higher mobility times in OT group (203.0±7.15 s) as compared to control group (173.75±12.09 s) in the same experimental conditions, but no statistical significance was obtained during posthoc analysis (Fmobility (1,6)=4.33; p=0.082). When we analyzed struggling time and floating time statistical significant differences were obtained.

Also, we observed that struggling time decreases in OT treated animals (8.0±2.08 s), as compared to controls (21.5±3.77 s), in a statistical significant manner (F(1,6)=9.80; p=0.02). Similarly, we obtained several differences while comparing the floating time between the OT group (37.0±7.15 s) and the control group (66.25±12.09 s), but no statistical variation was registered (F(1,6)=4.33; p=0.08) (Figure 3.4).

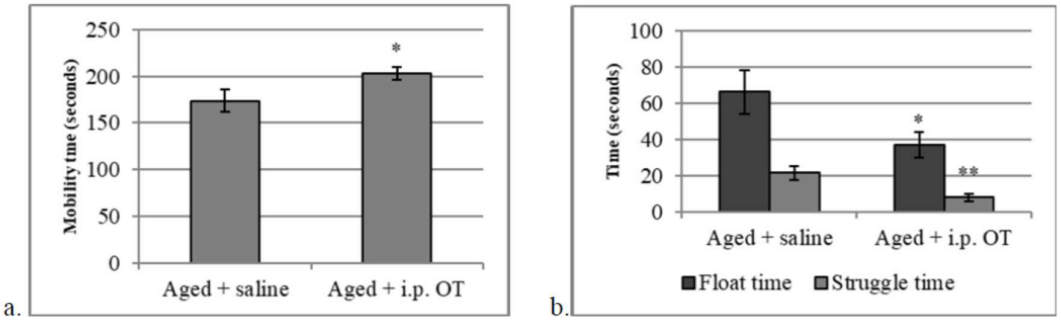


Figure 3.4. Effects of intraperitoneal OT administration on the parameters evaluated in forced swim test: a. mobility time (seconds); b. floating time and struggling time (seconds). The values are expressed as mean ± S.E.M. (n=5, per group) (* p=0.08 vs. saline; ** p=0.02 vs. saline).

During EPM administration, we observed that the OT-treated animals tendency to spent more time in the open arms (25.33±6.35 s) was significantly higher, as compared to control group (9.66±4.91 s) (F(1,6)=3.80; p=0.12). Also we observed that the time spent in the closed arms and center of the apparatus decreases after the OT administration, but not in a statistical significant manner (Fclosed arms (1,6) =0.52; p=0.49; Fcenter (1,6) =2.4; p=0.17). Although the time spent in the open arms was significantly different between the experimental groups, we observed that the head dipping behavior was practically of the same frequency (2.55±0.5) (Figure 3.5).

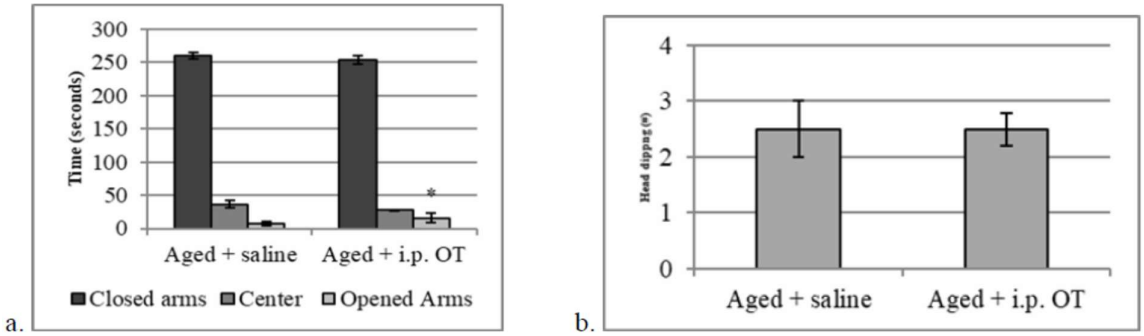


Figure 3.5. Effects of intraperitoneal OT administration on the parameters evaluated in elevated plus maze: a. time spent in the closed arms, center, and opened arms; b. number of head dipping behavior. The values are expressed as mean ± S.E.M. (n=5, per group) (*p=0.12 vs. saline)

In the typical test for assessing anxious behavior, the OFT, we mainly obtained differences regarding the time spent in the center of the open field paradigm apparatus, and the frequency and duration of the grooming behavior. In this way, we observed that the OT-treated animal spent more time exploring the center of the apparatus (12.75± 1.66 s), as compared to the control group (6.75±2.33 s) (F (1,6)=1.47; p=0.27). Also, we observed that the grooming behavior was significantly lower in frequency in the OT group (12.5±4.72 s) than in the control group (34.25±8.02 s) (F(1,6)=7.86; p=0.03) (Figure 3.6).

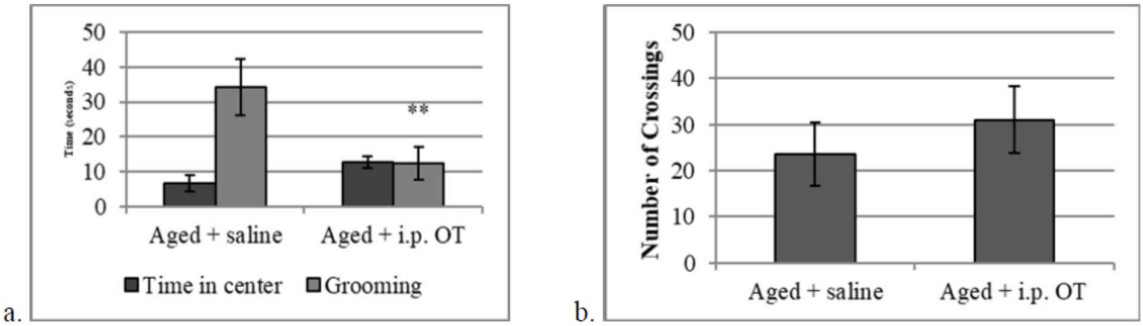


Figure 3.6. The effects of intraperitoneal OT administration on the evaluated open field test parameters: a. time spent in the center of the OFT apparatus and grooming behaviors; b. number of crossings. The values are expressed as mean ± S.E.M. (n=5, per group) (**p=0.03 vs. saline).

During the ST, we observed that the OT-treated animals exhibited a lower delay time until first grooming behavior (37.3±7.6 s), as compared to the control group (46.3±6.4 s), in the same experimental conditions. Also, we observed that the forced induction of grooming behavior resulted in decreased grooming behavior duration in OT group (58.5±27.8 s, as compared to 65.75±14.1 s in controls) (Figure 22). However, none of the presented variations were statistically significant (Fdelay (1,6)=1.32; p=0.29; Fgrooming time (1,6)=0.05;p=0.82). The effects of intraperitoneal OT administration on the evaluated Splash test parameters > latency time until first grooming, and grooming time. The values are expressed as mean ± S.E.M. (n=5, per group) (Figure 3.7).

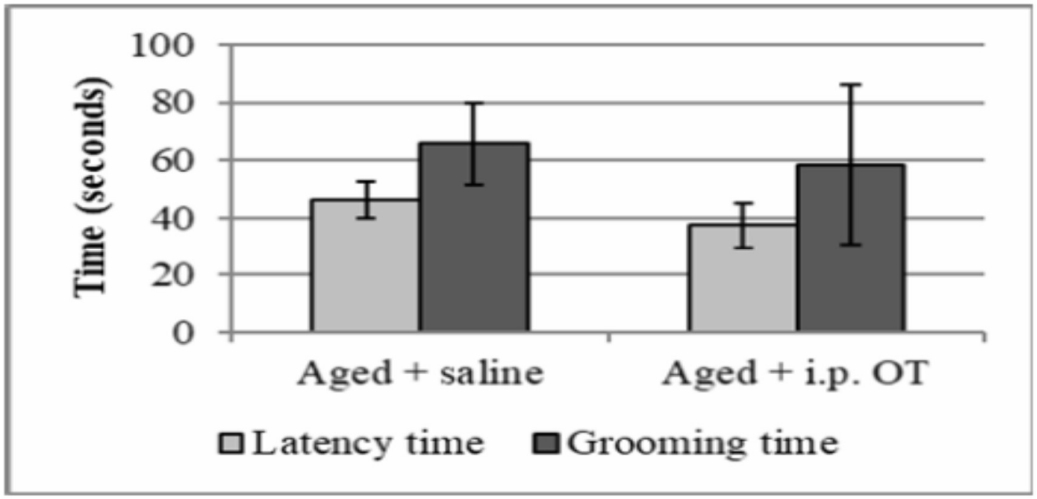


Figure 3.7. The effects of intraperitoneal OT administration on the evaluated Splash test parameters > latency time until first grooming, and grooming time. The values are expressed as mean ± S.E.M. (n=5, per group)

When we administered the TCT, we observed several differences regarding the time spent in the inhabited room (during first test phase), preferences for the stranger animal (contact duration with the familiar animal versus stranger animal), and anxiety behaviors when exposed to social stimuli (grooming and freezing behaviors).

While Crawley's test offers reliable data on sociability and social preference, it is important to consider that the measured social behavior is independent of general mobility (during all three phases), since the evaluation is made based on the contact duration rather the number of contacts with the animals quantified by other sociability tests (Takeuchi et al. 2011).

During the sociability test, which represents the first phase of the test, we observed that the OT-treated animals preferred to spend more time in the inhabited room (317.5±21.5 s) as compared to control group (195.0±10.2 s) ($F(1,8)=15.42$; $p=0.007$). Regarding the social preference, we observed also that OT group recorded higher contact duration with the stranger animals (56.75±10.3 s), than with the familiar ones (18.0±366 s) ($F(1,8)=17.61$; $p=0.003$).

The sociability and social preference in the control group were increased, but not in a statistical significant manner. Post-hoc analysis revealed significant differences regarding the mentioned parameters. In the same paradigm, we also measured the anxiety markers associated to the social stimuli.

Therefore, we observed that the grooming behavior was less frequent in OT-treated animal, as compared to the control group ($F(1,6)=2.12$; $p=0.2$). Also, differences were observed in regarding the freezing behavior, but no statistical significance was obtained ($F(1,6)=1.19$; $p=0.33$) (Figure 3.8).

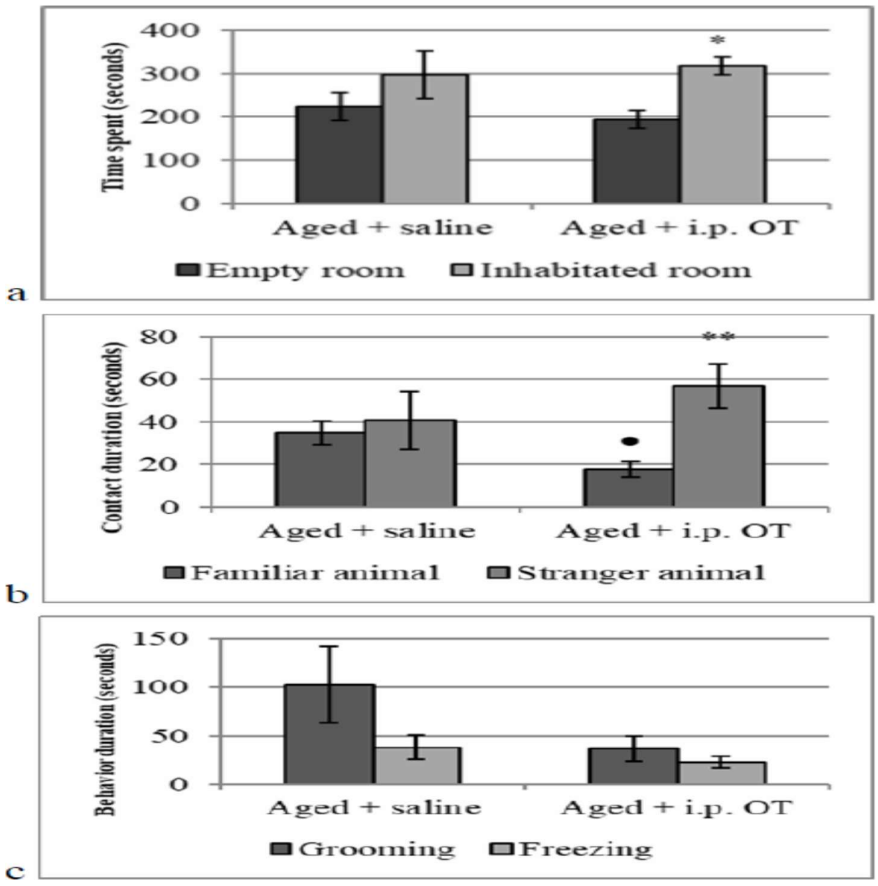


Figure 3.8. The effects of intraperitoneal OT administration on the evaluated three-chambered sociability test parameters: a. time spent in the empty and inhabited rooms (during phase I); b. time spent in the presence of the familiar and stranger animals (during phase II); c. grooming and freezing behaviors durations. The values are expressed as mean ± S.E.M. (n=5, per group) (*p=0.007, inhabited room vs. empty room; **p=0.01, familial animal vs. stranger animal; ● p=0.06 vs. saline).

**Discussion**

Thus, in this particular study, we investigated the effects of intraperitoneal administration of OT on the behavior of old animals, as compared to age and sex-matched control group. Now it is generally known that OT possesses high potential for social behavior enhancement (Lukas et al. 2011), but few information is available on its effect in memory, learning, and affective behavior of aged individuals. As stated before, the main known OT effects based on its hormone and neuropeptide properties include several physiological reproductive processes modulation and some behavioral implications such as maternal instinct, social bonding and mating, empathy, and mood enhancement (van Leengoed et al. 1987, Bick & Dozier 2011, Sheng et al. 2013).

In fact, our results provide evidence regarding the memory, mood and social behavior changes induced by intraperitoneal OT administration. The fact that peripheral OT administration leads to observable changes at central levels suggests that intraperitoneal route of administration may also be as effective as the intranasal route.

However, several changes in pharma kinetics were observed by comparing OT levels and peaks in brains and plasma of OT-treated rats (Neumann et al. 2013). In this way, although

intranasal OT administration was proved more efficient, intraperitoneal OT administration could cause rapid peak levels in brain and plasma during the first 30 min after treatment. In spite that, we showed that intraperitoneal OT administration also provides sufficient transport and interactions so that behavior changes are obtained.

Moreover, following OT administration, we observed a mild increase of the memory related behavioral parameter from the YMT leading to the suggestion that it may cause some memory changes.

Kunchiulia et al. (2010) showed that OT administration before stress exposing does not cause memory impairment prevention. Also, Sun-Young Lee et al. (2015) reports decreased cumulative search error, higher swim speed, and increased time in target annulus in OT treated rats, as compared with control group in the Morris water maze memory test.

These findings together with our results suggest that OT may influence hippocampal activity and memory acquisition in rats. However, our data showed no significant modification regarding the immediate working memory, as compared to our control group.

Despite that, we observed that OT administration resulted increased total mobility time in FST. Also regarding general mobility stand the results for number of crossings in the OFT paradigm (Figure 4.b) which exhibit a similar trending. These results show that OT may be involved in an anxiolytic pathway of spontaneous stress exposure relief (caused by exposure to water in FST or open field in OFT). Also, Yan et al. (2014), while modeling depression in rats via intra cerebro-ventricular OT receptor antagonist administration, obtained low immobility times in OT-treated rats, as compared with controls.

Moreover, the fact that OT administration decreases struggling behavior duration (in FST), and increases the time spent in the open arms of the EPM clearly suggests that OT exhibits a strong anxiolytic effect. However, no difference was observed in regarding the head dipping behavior, also considered an important behavior in assessing anxiety levels.

Even in that circumstance, it seems that OT had a more important effect on anxiety like behaviors reduce since freezing behavior was not observed almost in any of the OT-treated group. Moreover, we observed that the time spent in the center of the EPM was prolonged in OT-treated animals as compared to controls meaning that the OT treated animals were prone to exploration (a nonanxious behavior).

Also, we observed that the grooming behavior, considered an anxiolytic behavior, used to relief stress, was reduced in duration in OT-treated animals in both EPM and TCT as a result to environmental and social stress exposure.

In a similar way, the freezing behavior in social environment losses its intensity after OT administration leading to the suggestion that OT may also relief stress in social stimuli conditions. Another interesting aspect was observed while observing grooming behavior in ST. By contrast to noninduced grooming, which is an anxiolytic behavior, induced-grooming stands for depressive behavior evaluation.

In this way, by observing that delay time until first grooming in ST was considerably lower in OT-treated animals leads to the conclusion that OT also exhibits anti-depressant effects. These effects were also demonstrated by the low floating times observed in OT-treated animals meaning that the renunciation tendency was considerably decreased when exposed to OT.

As other authors reported, we also observed important improvements in social behavior after OT administration. In this way, we observed that the animals which received OT were prone to explore the inhabited room of the TCT during the first test phase meaning that the animals chose to spent time in a social environment rather than in isolation. Furthermore, during the second phase of the test, we observed that the OT-treated animals expressed the same social curiosity as compared to the first phase tendencies towards the stranger animals in the test. This leads to the conclusion that OT pays important role in sociability and also in social preferences.

In conclusion, we observed that short-term intraperitoneal OT administration as studied in aged rats shows important effects on anxiety a depressive behavior, and also in social interactions. In this way, increased mobility and decreased anxiety behaviors were reported for the aged intraperitoneal OT-treated animals, as compared with controls, during FST and OFT, and respectively FST, EPM, and OFT.

Also, significant differences were obtained regarding the social behavior of the intraperitoneal OT treated animal as compared to control group, the animals showing increased sociability and social preference for the stranger animal in TCT. However, no significant effects on the working memory (assessed as spontaneous alternation in YMT) were observed, suggesting a more predominant prosocial effect of OT.

Regarding the importance of oxytocin in most of the neuropsychiatric disorders and the latest development in the context of the oxidative stress status modifications in this matter, we could mention for example that lately there is an increased interest in understanding the mechanisms involved in the pathological manifestations from the main neuropsychiatric disorders (Ciobica et al., 2016).

In this way, oxytocin is a complex molecule involved in a variety of biological processes at both the central and the peripheral level. Although its role was initially associated almost exclusively with birth and breastfeeding, recent studies are suggesting that in fact oxytocin could be involved in many other physiological and neuropathological/ psychiatric processes (Ciobica et al., 2016).

In the same way, the aforementioned research group showed in 2018 that oxytocin administration could actually improve memory, anxiety and some oxidative stress parameters in a methionine-induced rat model of schizophrenia (Padurariu et al., 2018).

Thus, in the last few years, these results have generated a lot of discussion about the possibility of translating oxytocin's effects into therapeutic applications with special focus on autism, schizophrenia, anxiety, depression and even frontotemporal dementia or Huntington disease (basically where the social cognition and emotion recognition are affected) (Cheong et al., 2020).

In fact, most of the studies regarding the effects of (intranasal) oxytocin in the neuropsychiatric disorders, as we also described, are mainly characterized by controversial and not-so-easy to reproduce results.

For example, in autism, it was found that there are various deregulations of plasma oxytocin levels in children with autism spectrum disorder (ASD), when compared to age matched controls and also the infusion of synthetic oxytocin (Pitocin) reduced the frequency of repetitive behaviors, as compared to placebo (Hollander et al., 2018), while on the other side other authors and studies had failed to found therapeutic effects of oxytocin in treating various pathological manifestations in autistic patients [5-a nature group complex study].

Similar controversies were also observed in anxiety, since some authors reported that the administration of intranasal oxytocin in small samples of patients with anxiety disorder is somehow reducing some specific anxiety manifestations, while other studies showed that the addition of oxytocin to exposure therapy did not enhance treatment outcomes for anxiety, and especially for the long-term management of this disorder (De Cagna., 2019).

Inconsistent results were also presented in the case of schizophrenia, since some authors reported that the lowest CSF oxytocin levels were found in normal subjects, while significantly higher levels were found in schizophrenia patients without antipsychotic treatment and the highest oxytocin levels were reported in schizophrenia patients treated with antipsychotic drugs (Liu et al., 2019).

# SECTION I: CHAPTER 4.
# Biological mechanisms of affective disorders

**Introduction**

No efficient specific treatment has been yet developed, the therapy relying only on symptomatic alleviation (Hirth, et al. 2011, Rouault at al. 2013, Ghavami et al. 2014). This is also the case for the affective disorders or mood disorders, which are a group of well-studied related psychiatric disorders which have common socio affective features and can accompany unipolar, bipolar, or schizoaffective syndromes (Hudson et al. 1990).

The correlation between the affective disorders and the almost ubiquitous pathological oxidative stress can be described in a multifactorial way, as an important mechanism of central nervous system impairment. Whether the obvious changes which occur in oxidative balance of the affective disorders are a part of the constitutive mechanism or a collateral effect yet remains as an interesting question. However, it is now clear that oxidative stress is a component of these disorders, being characterized by different aspects in a disease-dependent manner.

It seems that several cellular and molecular features of the affective disorders are quite similar, disregarding the specific clinical symptomatology. In this way, one of these aspects is oxidative stress status, which seems to be implicated in most of the different affective disorders, since it has been shown that increased oxidative damage occurs quite often in depression (Michel et al. 2012, Black et al. 2015, Vavakov ´et al. 2015) anxiety (Bouayed, 2009), bipolar disorder (Smaga et al. 2015, Andreazza et al. 2008) panic disorder (Ersoy et al. 2008, Gul et al. 2013) and also in obsessive compulsive disorder (Kandemir et al. 2013).

Oxidative stress can be easily defined as the condition arising from the imbalance between toxic reactive oxygen species and the antioxidant systems. Shortly, the most studied ROS are the superoxide anion (O2−), hydroxyl radical (HO−), hydrogen peroxide (H2O2), nitric oxide (NO), peroxyl (ROO−), and reactive aldehyde (ROCH), while on the other side these reactive species are dealt with by the body in several ways, including the usage of the antioxidant enzymes system. Thus, SOD catalyzes the conversion of superoxide radicals to hydrogen peroxide, which is then converted into water by GPX, and catalase, as our group previously demonstrated on different occasions through most of the neuropsychiatric disorders (Padurariu et al. 2010, Stefanescu et al. 2012, Ciobica et al. 2011, Hritcu et al. 2011, Hritcu et al. 2013)

Still, there are a lot of controversies regarding the relevance of the oxidative stress status in most of the affective disorders and despite the fact that most of the studies are showing that the affective disorders development can be correlated to increased oxidative levels, there are various studies stating that oxidative stress is not linked with the mood changing tendencies.

Thus, next we decided to describe the way in which oxidative stress is involved in the affective disorders development, by focusing on the main oxidative stress markers that could be used mechanistically and therapeutically in these deficiencies, the genetic perspectives, some antioxidant approaches, and the relevance of some animal models studies in this context.

## 4.1: Oxidative Stress Implications in the Affective Disorders: Main Biomarkers, Animal Models Relevance, Genetic Perspectives, and Antioxidant Approaches

### A. Background

As we mentioned before, lately the interest regarding inflammation and oxidative stress as important pathogenic pathways in psychiatric pathology increased. In fact, a strong link between the inflammatory, oxidant, mitochondrial, and apoptotic markers versus the cognitive decline has been developed and theorized (Halliwell et al, 2011).

It seems that all these pathological background features are somehow molecularly linked, leading to a complex interaction between the cellular/molecular control and causes effects conditioning. This is why, generally speaking, most of the neuropsychiatric disorders causes that are leading to the known and seen symptoms are a rather problematic matter to determine and to successfully discriminate from other collateral features. In this way, the neuropsychiatric diseases still remain partly unknown due to a multifactorial background.

### B. Published article in this field

Regarding this field we published the following review article:

Ioana Miruna Balmus, Alin Ciobica, Iulia Antioch, **Romeo Dobrin**, Daniel Timofte : Oxidative Stress Implications in the Affective Disorders: Main Biomarkers, Animal Models Relevance, Genetic Perspectives, and Antioxidant Approaches, Oxidative Medicine and Cellular Longevity, Volume 2016, Article ID 3975101, 25 pages, **IF 2016 = 4,59**

#### Introduction

The correlation between the affective disorders and the almost ubiquitous pathological oxidative stress can be described in a multifactorial way, as an important mechanism of central nervous system impairment. Whether the obvious changes which occur in oxidative balance of the affective disorders are a part of the constitutive mechanism or a collateral effect yet remains as an interesting question. However, it is now clear that oxidative stress is a component of these disorders, being characterized by different aspects in a disease-dependent manner.

Oxidative stress can be easily defined as the condition arising from the imbalance between toxic reactive oxygen species and the antioxidant systems. Shortly, the most studied ROS are the superoxide anion (O2−), hydroxyl radical (HO−), hydrogen peroxide (H2O2), nitric oxide (NO), peroxyl (ROO−), and reactive aldehyde (ROCH), while on the other side these reactive species are dealt with by the body in several ways, including the usage of the antioxidant enzymes system. Thus, SOD catalyzes the conversion of superoxide radicals to hydrogen peroxide, which is then converted into water by GPX, and catalase, as our group previously demonstrated on different occasions through most of the neuropsychiatric disorders (Padurariu et al. 2010, Stefanescu et al. 2012, Ciobica et al. 2011, Hritcu et al. 2011, Hritcu et al. 2013)

The main spectrum is constituted of several psychiatric pathological conditions which occur in different combinations determining variable social or affective behavior classified as mood impairments. The mainly known affective disorders are depressive disorder anxiety disorder, obsessive-compulsive disease, panic disorder and posttraumatic stress disorder (Figure 4.1).

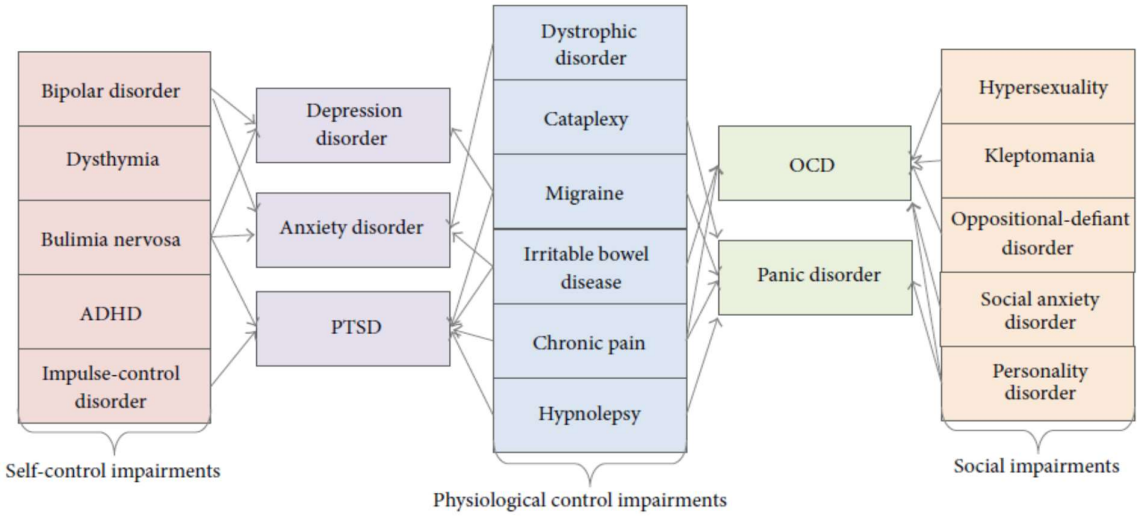


Figure 4.1. Affective disorders vertical classification (ADHD: attention deficit and hyperactivity disorder; PTSD: posttraumatic stress disorder; OCD: obsessive-compulsive disorder). Some of the symptoms for the affective disorders are quite distinct between the affective variants groups, while the main affective disorders (ANX, DD, PTSD, OSD, and PD) are more likely symptom combinations of the groups. Therefore, ANX, MDD, and PTSD exhibit both self-control discrepancies, as observed in bulimia, impulse-control impairment, or attention deficits, and physiological control alterations, such as irritable bowel disease, frequent migraines, or chronic pain. Furthermore, on the opposite side stand OCD and PD, which exhibit mainly social impairments, such as oppositional-defiant behavior, social anxiety, and different personality discrepancies, as well as physiological impairments. In this way, it seems that the major affective syndromes can be classified given the general symptomatology tendencies in two groups: self-control-associated syndromes (DD, ANX, and PTSD) and social-hurdle syndromes (OCD, PD) (based on Hudson et al. 2003).

It is important to mention that various tissues have different susceptibilities to oxidative stress. In fact, the correlation between the oxidative stress status and affective disorders development could arise from the vulnerability of central nervous system to oxidative damage.

Oxygen related free radicals and reactive species are both produced by the body, primarily as the result of the aerobic metabolism (Bild et al. 2013). In the intra and extraneuronal environment, these molecules have also important functions, such as synaptic plasticity and memory regulation (Massaad et al. 2011). More than that, the CNS tissues are rich in lipid molecules which are an easy target for oxidation reactions of the pathways in which they are involved (Halliwell et al. 2011). In addition, the metabolism of some neurotransmitters is also based on redox potential transmission (Lehtinen at al. 2006).

Considering these aspects, it has been shown, for example, that neuroinflammatory pathways activation together with CNS oxidative and nitrosative stress could play an important role in the depressive disorder pathophysiological background (Padurariu et al. 2010). Interestingly, it seems that ROS and RNS can cause immune response aberrations alongside

molecular membranes structural alteration leading to immunogenic properties that could alert the immune system in the presence of oxidized fatty acids. This would be pathway through which DNA, proteins, lipids, and mitochondria damages can lead to the dysfunctions observed in depressive disorders (Shao et al. 2008). Moreover, mitochondrial dysfunctions have also been reported in the context of ROS and RNS overproduction described in BD. As mitochondria are the most active organelles in ROS/RNS production, it seems that they also contribute to affective disorders pathological mechanisms. It has been shown that, in BD, the occurrence of mitochondrial DNA mutations and the occurrence of other mitochondrial diseases are rather high (Clay et al. 2011). This could be the reason why mitochondrial metabolism correction could actually result in some alleviation of BD symptomatology (Hovatta et al. 2010).

Regarding oxidative stress and anxiety-related phenotypes a strong bound between oxidative stress and anxiety has been observed (Kumar et al. 2013). In this way, using genetically ANX-predisposed mice strains, the correlation between several antioxidant enzymes such as glutathione reductase-1 or glyoxalase-1 hyperactivity and intense anxiety behavior has been described. Since then, many of the studies showed that anxiety is a GABAergic and serotonergic modulated condition (as reviewed by Cyranna et al. 2006). Thus, it seems that both GABA and 5-HT are involved in the modulation of anxiety responses in the brain. Regarding the GABA release modulation, it seems that in anxiety the most important aspect is the 5-HT capacity to modulate the excitability of GABAergic interneurons.

Several detailed studies on hippocampal response to serotonin stimulation via dorsal raphe fibers showed that serotonin specifically targets a subset of hippocampal interneurons involved in GABA B-mediated feed forward inhibition [36]. Moreover, GABA-A receptor agonists were showed to inhibit untrained anxiety reactions. In this way, intrahippocampal infusions of glutamatergic, serotonergic, and cholinergic compounds are thought to produce reliable antianxiety effects (Engin et al. 2007). Also, GABA release modulation has been observed in other brain areas involved in anxiety behavior regulation, such as dentate gyrus, which plays important roles in generating contextual memories of fear; entorhinal cortex, which modulates contextual fear memory extinction; piriform cortex which consists in amygdala, the fear center, frontal cortex involved in subcortical fear response, and anterior cingulate cortex-remote contextual fear memory (Distler et al. 2005).

Furthermore, a clear connection between fear response and oxidative damage was established being given that glyoxalase 1 cytotoxic substrate, methylglyoxal, is one of the well-known GABA agonists and also a potent modulator in oxidative stress and apoptosis (Hovatta et al. 2005). In this way, Hassan et al. (2014) demonstrated a close interaction between brain antioxidant system genes expression (glutathione reductase 1 and glyoxalase 1) and anxiety-like behavior. Also, the link between oxidative stress and emotional stress, such as fear and phobia, is based on the oxidative impairment caused in the brain by the imperfect gene expression that leads to oxidative and enzymatic unbalance.

However, the modulation properties of ROS on glutathione reductase 1 and glyoxalase 1 genes expression are yet unclear, as Hassan based their conclusions on lentiviral-modulated gene expression. The fact that antioxidant enzymes' genes overexpression was observed in fear-controlling brain areas in the absence of oxidative stimuli leads to the conclusion that there might be other stress-controlled mechanisms that are involved in anxiety-like behavior occurrence.

Moreover, Hassan et al. (2014) showed that oxidative stress may actually be the leading cause of ANX, while Masood group (Masood et al. 2008) found that glutathione synthesis inhibition may induce hippocampal and amygdala oxidative stress, leading to the assumption that hippocampal oxidative stress and anxiety could be indeed connected.

Regarding panic disorder, the latest studies also describe a strong connection between antioxidant enzymes activity and panic symptomatology (Salin et al. 2014, Cneginz et al. 2015). For instance, a certain phenotypic formula in antioxidant defense may be associated with gender specific panic development. Thus, the Pro198Leu polymorphism of the glutathione peroxidase 1 gene seems to participate in the development of anxiety-like behavioral phenotypes. Also, panic disorder was newly characterized as an anxiety spectrum disorder due to the similarities between the oxidative mechanisms (Liu et al. 2014).

Also, in obsessive compulsive disorder, it seems that mitochondrial dysfunction could be an important etiopathogenic mechanism. (Orhan et al. 2012). Moreover, a correlation between mitochondrial disorders and oxidative stress in obsessive compulsive disorder was revealed due to a genetic variability of manganese-dependent superoxide dismutase and a small mitochondrial protein (Pagano et al. 2014). More than that, an oxidative imbalance has been reported in these patients, but unfortunately the exact pathway in which oxidative stress is involved is still partially unclear (Gul et al. 2013).

The exact correlation between major depressive disorder and oxidative stress is also a major concern, since a close connection between glutamatergic hyperactivity and depression has been postulated (Deschwanden et al. 2011). Glutamate is the predominant excitatory neurotransmitter being responsible for synaptic plasticity, learning, memory, and locomotion. The glutamatergic system naturally regulates the glutamate concentrations in the synaptic shaft via both neuronal and glial receptors. Several stressful stimuli lead to excessive release of glutamate into the synapse that can cause glutamatergic hyperactivity, neurotoxicity, and cell death when neuronal receptors extendedly activated. Following excessive glutamate release, a decrease in brain GABA is observed since glutamate is being used by the brain to synthetize GABA . Standing several brain imaging studies are also supporting this hypothesis, which showed that acute depression is associated with low prefrontal and occipital cortex GABA concentrations (Petroff et al. 2002).

Thus, alongside presynaptic downregulation of GABAergic system, and therefore GABAergic neurons activity reduction, GABA-A receptor function may be impaired (Esser et al. 2007). Furthermore, GABA-A mediated neuronal inhibition induced by pre- and postsynaptic sites interaction with ROS may also contribute to the development of neuronal damage leading to neurotransmission impairments (Sah et al. 2002). Also, several studies suggest that the glutamatergic system dysfunction is obvious due to the exceptional efficiency of ketamine in depression treatment.

More specific studies on this aspect showed that increased NMDA receptor activity and glutamatergic synapse impairment are leading to depressive behavior when localized in the prefrontal cortex. In this way, several studies managed to demonstrate equally hyperactive NMDA receptors and glutamatergic synapses both in depressive patients and in animal models of depressive behavior (Wang et al. 2014, Li et al. 2011).

Thus, increased glutamate levels could lead to free radical formation by xanthine oxidase, for example, and further production of oxygen radicals and oxidative neurological

damage. Also, nitric oxide related toxicity caused by peroxynitrite formation in NO and superoxide anions reaction results in microfilament tyrosine residues nitration (Sin et al. 1990). At the same time, Mg-SOD activity, which is positively modulated by ROS accumulation, could inhibit the mitochondrial respiratory chain and the glutamate transporter and therefore lead to glutamate-induced neurotoxicity (Aoyama et al. 2000).

However, major depression has been characterized as a progressive stage-related process of neurodegeneration caused by apoptosis, reduced neurogenesis or neuronal plasticity, and increased autoimmune responses. Thus, increased oxidative stress markers and neuroinflammation have been reported in the blood of depressed patients (Herken et al. 2007) but the molecular pathways through which impaired redox homeostasis interacts with the immune-inflammatory system in relation to major depression are still not clear. Without any doubt, there are still a lot of controversies regarding the relevance of the oxidative stress status in most of the affective disorders and despite the fact that most of the studies are showing that the affective disorders development can be correlated to increased oxidative levels (as we showed for most of the shortly described studies above), there are also some distinct studies reporting that oxidative stress may not be linked in any way, for example, with posttraumatic stress disorder (Ozdemir et al. 2015, Lang et al. 2015).

Therefore, in this subchapter we decided to describe the way in which oxidative stress is involved in the affective disorders development, by focusing on the main oxidative stress markers that could be used mechanistically and therapeutically in these deficiencies, genetic perspectives, and antioxidant approaches, as well as the relevance of some animal models studies in this context.

**Material and Methods**

Regarding the methodology, the information gathered for this review was searched in the main available databases (e.g., ScienceDirect, PubMed/ Medline, Embase, and Google Scholar). Concerning the relevance of some oxidative stress markers affective disorders, currently, many hypotheses are describing the affective impairments. Depression development, for example, relies on psychological, psychosocial, hereditary, evolutionary, and biological combined factors. In this way, most of the theories rely on the monoamine hypothesis which states that serotonin, norepinephrine, and dopamine can assist the development of depression in a concentration-dependent manner.

**Results and Discussions**

Serotonin is thought to modulate other neurotransmitter systems and therefore any changes in its concentration may lead to unusual or aberrant neurotransmission (Lang et al. 2013). Low serotonin levels are promoting low norepinephrine levels which could lead to depression (Barlow, 2005). Correlated to this, a widely known hypothesis stated that certain monoamine neurotransmitters can lead to affective impairments: norepinephrine deficits may be related to alertness and energy loss leading to anxiety, attention deficits, and loss of interest in life, while serotonin deficits is related to anxiety, obsessions, compulsions, and dopamine system impairment to attention and motivation loss (Shah et al. 1999). Still, many limitations of the monoamine hypothesis led to the conclusion that depression may be rather complex almost certainly multifactorial (Nutt, 2008, Krishnan et al. 2008).

In this way, oxidative stress and its main markers could represent some viable solution for the understanding and for a better management of some affective disorders. In fact, clinical trials were the primary source of evidence that oxidative stress could be implicated in the pathogenesis of the affective disorders. Moreover, it was shown that the mood stabilizers and antidepressant therapies possess high antioxidant potential (Behr et al. 2012). Also, other studies showed that some oxidative stress markers are normalizing during or after the specific therapy applied for the affective episodes, suggesting that antidepressants could actually reduce oxidative stress levels (Sarris et al. 2011). In this way, it was actually shown that several antidepressants such as tianeptine (Quevedo et al. 2012), escitalopram (Shalabi et al. 2009), venlafaxine (Eren et al. 2007) or mirtazapine (Tok et al. 2012) could exert antioxidant effects.

However, there are still a lot of controversies about this subject, since some authors such as Bilici et al. showed that the administration of some selective serotonin reuptake inhibitors (SSRIs) for 3 months is generating a normalization in levels for some oxidative stress markers such as some antioxidant enzyme activities and lipid markers (Bilici et al. 2001) or the Gałecki group, which demonstrated that fluoxetine given together with acetylsalicylic acid is decreasing the oxidative stress levels in patients with major depression disorder (Galecki et al. 2009) while on the other side the group of Sarandol stated no significant modifications in the oxidative stress levels after venlafaxine and sertraline administration for 6 weeks and/or the same Gałecki research group, which showed no modifications at all in the levels of some oxidative stress markers such as the glutathione peroxidase after 3 months of fluoxetine treatment (Sarandol et al. 2007, Galecki et al. 2009).

In this way, one possible explanation for this lack of homogenous results could be represented also by the dosage of antidepressant used, since, for example, 40mgof fluoxetine could exert some antioxidant effects (Kilm et al. 2007) which are not visible in other studies that used 10 or 20 mg, as in the aforementioned Gałecki et al. study (Ozcan et al. 2004) (as also described in [Stefanescu et al. 2012]).

In fact, in depression, while most of the authors have generally described decreased levels of GPX and increased levels of MDA, as a lipid peroxidation marker (Sarandol et al. 2007, Ozcan et al. 2004) there are also controversies regarding the specific activity of some antioxidant enzymes such as SOD, which was reported to be decreased in patients with depression (Herken et al. 2007) showing no significant modifications when compared to controls or a significant increase in most of the studies (Sarandol et al. 2007, Galecki et al. 2009, Khanzode et al. 2003, Kodydkov´a et al. 2003).

As we also mentioned before when we described the levels of SOD in some neuropsychiatric disorders (Padurariu et al. 2010) this could be perhaps explained by the fact that SOD represents the first enzyme to get in contact with the free radicals and its increase may suggest some compensatory actions. However, when the specific activity of both SOD and GPX us decreasing, this will lead to an accumulation of hydrogen peroxide that will stimulate in a cascade of the lipid peroxidation processes and protein oxidation, which could explain some pathological manifestations observed in these disorders.

In this way, it seems that lipid peroxidation is an important component of the oxidative stress status observed in depression (Palta et al. 2014). In fact, our group showed that subclassifying depression into different stages, based on chronicity (e.g., first episode versus recurrent depression), can actually predict significant differences in the levels of some lipid

peroxidation markers such as MDA and also in the specific activity of the main antioxidant enzymes such as SOD and GPX (Stefanescu et al. 2012).

Thus, perhaps an increased production of oxygen and nitrogen reactive species in these patients could generate a rapid consumption of the plasmatic antioxidants. Thus, in a so called vicious cycle in the various staging of these affective pathologies, we could face an inadequate antioxidant enzymatic activity incapable of counteracting increased concentrations of free radicals and inflammatory processes, as we will show immediately.

Moreover, similar facts were showed by our group in the case of the mild cognitive impairment and AD patients (Padurariu et al. 2010) so the aforementioned aspects could represent perhaps an important pathway in the development of these neuropsychiatric disorders. However, Dimopoulos et al. group (Dimopoulos et al. 2008) showed that plasma levels of isoprostane-8-epi-prostaglandin F2 alpha gets unusually high in elder patients with depressive symptoms. Moreover, (Müller et al. (2015) propose a new marker of oxidative stress, based on the fact that the brain membrane lipids are very important in depressive and anxiety disorders progression.

In this way, it seems that low polyunsaturated fatty acids (PUFA) levels can be correlated with low antioxidant protection and an increased n-3 PUFA supply may reduce mood-related behaviors. In fact, it seems that omega-3 fatty acids may actually alleviate some depression-related effects (Wilczy´nska, 2013). In this way, as we will insist on the last chapter of this review, dedicated to the possible antioxidant therapeutic approaches in most of the affective disorders treatment, several recent studies also showed that eicosapentaenoic acid supplementation was actually quite effective against primary depression (Sublette et al. 2011). Moreover, it has been also observed that GPX homologues could exert some antidepressant effects (Zhao et al. 2008, Posser et al. 2009) while Brown et al. (2009) demonstrated that lipid peroxidation, DNA/RNA damage, and nitric oxide levels could be relevant markers in the depression pathology.

As we mentioned before, several recent studies such as the one of Black et al. in 2015 (Black et al. 2015) revealed that both 8-hydroxy- 2-deoxyguanosine (8-OHdG) and F2-isoprostanes are increased in depression, suggesting a strong implication of inflammation and oxidative stress in its pathological mechanisms. Moreover, this recent finding supports the hypothesis that increased metabolic stress is present in depression contributing to its high somatic morbidity and mortality. In fact, there are opinions in the literature that depression could be considered an inflammatory disorder, as judged mainly by the increased levels of the proinflammatory cytokines, such as interleukin-1b, interleukin-6, or tumor necrosis factor-alpha (Maes et al. 2012). There is also a “vicious cycle” of pathogenic manifestations in this case, considering that depression could be correlated to an increased production of proinflammatory cytokines that, in turn, would lead to increased oxidative stress (Maes et al. 2008, Catena-Dell’Osso et al. 2011) while also decreased levels of antioxidants/antioxidant enzymes could generate increased inflammatory response (Kobrosly et al. 2010).

Brain antioxidant deficiencies also contribute to an oxidative damage which was observed in bipolar depression. In this way, glutathione was found in low concentrations in the prefrontal regions of bipolar patients (Benes et al. 2006) while downregulations of important antioxidant enzymes (such as superoxide dismutase 1, glutathione peroxidase 4, and glutathione Stransferase) were observed in hippocampus (Hoffman, 2016). Moreover, considering that the

thiobarbituric reactive substances (peroxidized species of lipids or lipid complexes) can easily change protein conformations and therefore disturbing lipid messengers signaling systems (Poon et al. 2004, Bazan et al. 2005) some authors found that, in bipolar disorder, the oxidative stress to lipid structures could actually increase in a stage-dependent manner, disregarding the mood episode Dr¨oge et al. 2007, Berk et al. 2007).

On the other hand, as the nitric oxide is involved in the excessive release of glutamate and abnormal reactions to thiol proteic groups, it seems that the role of glutamate-induced oxidative stress via nitric oxide could be also extremely relevant in bipolar depression (Berk et al. 2007). In addition, groups such as the one lead by Grande et al. (2012) or Vieta et al. (2013) suggested that alongside the progression of the bipolar disorder, several markers such as neurotrophins and inflammatory cytokines (tumor necrosis factor-alpha (TNFa)) could be well correlated to the pathological evolution of the disorder. Moreover, Kapczinski et al. (2009) stated even from 2000 that TNFa levels could represent an important marker in the bipolar disorder staging.

Also, it was stated that the lipid peroxidation processes could represent an important biomarker in bipolar disorder progression, together with 8-OHdG, which can cause improper translation and proteic aggregation (Shan et al. 2003) and with 5-hydroxymethylcytosine (5-HmC) (Matarese et al. 2011). Moreover, it seems that oxidative stress can alter brain activity through similar mechanisms, but with different visible behavioral manifestations. In this way, glyoxalase 1 is an enzyme which protects against carbonyl stress, reaction controlled by glutathione as a cofactor for this enzyme (Thornalley et al. 2003).

On the ground that glutathione reductase 1 and glyoxalase 1 are antioxidant factors which are highly correlated to anxious behavior, many studies have been conducted in order to find the nature of this correlation. Thus, Hovatta et al. (2010) showed that overexpression of the glutathione reductase 1 and glyoxalase 1 gene leads to anxiety-like behaviors, while inhibition of glyoxalase 1 expression produces only low intensity anxiety like behaviors. Also, based on the fact that excessive ROS accumulation induces overexpression of these genes and therefore intense activity of the enzymes, it can be speculated that they could regulate anxiety. However, there are also controversial results in this area of research, since these findings were discordant with other studies which showed that glyoxalase 1may be a marker for the anxiety trait (Kr¨omer et al. 2005, Ditzen et al. 2006).

Also, mechanistically speaking anxiety could be related to low levels of gamma-aminobutyric (GABA) occurrence which is reducing brain activity (Dunlop et al. 2008). In this way, either overactivation or underinhibition can lead to cortical and limbic glutamate neurotransmission through N-methyl-D-aspartate (NMDA) receptors that is linked to an excess of stimulatory glutamate, calcium influx, or insufficient GABA or GABA receptor function deficits. Additionally, the research on the GABAergic system has been performed on panic disorder and OCD animal models and patients (Kuloglu et al. 2002) demonstrating also that oxidative metabolism can affect the regulation of anxiety behavior.

In this way, it has been shown that in oxidant conditions as due to the lipid-rich constitution of brain, lipid peroxidation increase which causes membrane fluidity impairment and probably impairments in receptors, enzymes, and ion channels functions (Valko et al. 2007) Therefore, it is quite possible that oxidative stress could alter neurotransmission, cell signaling, and therefore brain activity in these pathologies (Delattre et al. 2005).

### a. Oxidative Stress Implications in Some Animal Models of Affective Disorders

It is generally considered that the generation of various animal models is an extremely valuable tool in understanding the mechanisms behind a variety of specific diseases. Also, animal models are widely and efficiently used in the affective disorders research area, considering the obvious ethical constraints in using human subjects and the impossibility to control the human individual variability (Hoffman, 2016).

Of course, the animal models are not the perfect representation of the complex human diseases, especially considering the fact that psychiatric concepts such as self-esteem, recurrent thoughts of death (Rotzinger et al. 2010) or fear of losing control (Cryan et al. 2011) are not reproducible in this case. Instead, they are created to mimic certain characteristics of the disease or a behavioral dimension specific to that psychiatric pathology (e.g., affective disease in this case) (Lefter et al. 2014). Due to this fact, it is extremely important to correctly assess the specific affective spectrum behavior. In order to do this, there are many behavioral tests which can be successfully used (please see Figure 4.2 for a more complex example).

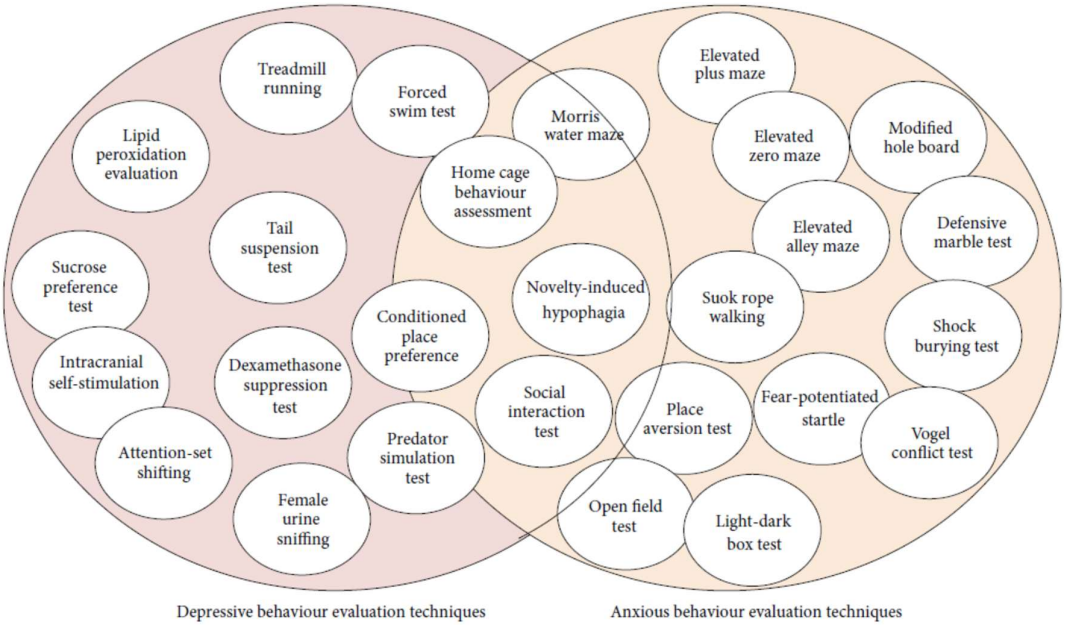


Figure 4.2. Behavioral tests battery used in depression/anxiety assessment (Dedic et al. 2011). Due to the fact that depressive and anxious behaviors are interconnected, and in some cases interdependent, it is very important for the difference of anxiety as a trait or as a symptom, for example, to be clearly defined. Thus, several evaluation techniques can only evaluate depressive behavior (the tests shown in the left side of the picture), being useful in determining clear depressive traits. On the opposite side the typical anxious behavior techniques stand which are meant to evaluate general and conditioned anxiety, while in the middle the depressive-related anxiety techniques stand, which can be used in both depression and anxiety evaluation. This aspect can be crucial when elaborating complex hypothesis regarding common symptomatology and behavior which leads to elucidating information in affective syndromes etiology.

Also, these animal models must fulfill several criteria to be validated. For this reason, they must be comparable to the human dysfunction in the aspects of symptomatology (face validity), treatment manners (predictive validity), similar causative neurobiological factors (construct validity), and common etiology (etiological validity) (Cz´eh et al. 2016). Another

aspect that must be met is repeatability between laboratories and various studies (Crabbe et al. 1999). Actually, new models are designed or the existing ones are improved due to the need of a higher accuracy. It is also the case of affective disorders modelling in animals considering ethological aspects, genetics, surgical procedures, chemical induction, or their combination resulting in a multitude of animal models. In this way, it seems that oxidative impairments observed in the animal models of affective disorders are somehow similar to the disparities found in humans.

Thus, Brocardo' team, for example, demonstrated the presence of increased levels of oxidative damage in a rat model of fetal alcohol exposure, in which they created the conditions of anxiety and depression-like behavior. They recorded significantly higher levels of lipid peroxidation and protein oxidation measure from the hippocampus and cerebellum, while physical exercising displayed protective effects in this matter and increased the rates of glutathione (Brocardo et al. 2012).

Also, it was showed that in bipolar disorder animal models there are various alterations for the protein oxidation markers, with the specific activity of SOD and CAT being increased and GPX activity decreased. Also, the levels of lipid peroxidation markers were found to be increased, which correlated to low rates of glutathione and vitamin C. Moreover, the administration of lithium and valproate in these cases was associated with a significant reduction for the lipid peroxidation processes in the hippocampus and the prefrontal cortex Frey et al. 2006, Andreazza et al. 2008).

In other several models of depression in rats it was showed that these animals exhibit alterations of some oxidative mechanisms in the form of glutathione levels depletion, decrease in GPX specific activity, lower levels of vitamin C, or increased rates of lipid peroxidation and nitric oxides (Eren et al. 2007, Pal et al. 1994). Another study also showed that lamotrigine, aripiprazole, and escitalopram exerted some protective effects against depression linked GPX, glutathione, and vitamin C deficiency and also decreased lipid peroxidation levels.

Moreover, from the aforementioned three drugs, it seems that lamotrigine was associated with the strongest antioxidant protective abilities (Eren et al. 2007). Also, we can mention here the study of Kumar group, which used an immobilization stress animal model of anxiety and proved that the six-hour time spent in restraint could considerably increase the brain concentrations of lipid peroxidation markers and nitrite in the animals. Furthermore, the same anxiety model had important effects on brain glutathione and catalase rates which were significantly decreased compared to the control group (Kumar et al. 2014).

Another animal model of affective disorder induced by ouabain (African plant derived toxic substance) single intracerebroventricular injection which actually could mimic the conditions of mania and that is course characteristic to the bipolar disorder resulted in increased thiobarbituric acid reactive substances (TBARS) and carbonyl levels especially in the frontal cortex and hippocampus area, while elevated SOD activity and reduced CAT specific activity were also reported in the aforementioned central areas (Valvassori et al. 2015).

Moreover, the same group showed that sodium butyrate (e.g., with the inhibition of Na+/K+-ATPase produced by ouabain) could counteract the oxidative alterations induced by specific toxin administration, through the reversal of the protein and lipid disturbances found in the hippocampus and prefrontal cortex of injected rats and by increasing CAT specific activity (Lee et al. 2003).

In addition, phenelzine, which is a monoamine oxidase inhibitor drug, showed great antioxidant defense potential, being capable of reducing the reactive oxygen species formation and the scavenging proprieties of hydrogen peroxide (Abdel-Wahab et al. 2011). Also venlafaxine, a drug from the selective serotonin reuptake inhibitors group, was able to reverse the deficits in glutathione (GSH) rates and also to decrease the levels of hippocampal MDA and nitric oxide (NO) induced by the specific stress depression tests such as forced swim test and tail suspension (Padayatty et al. 2003).

In addition, the well-known antioxidant ascorbic acid (Moretti et al. 2012) was reported to reverse oxidative damage in an induced model of depressive disorder, as compared to fluoxetine treated controls, by mainly increasing the specific activity of CAT and glutathione reductase. Oxidative unbalance was also demonstrated in anxiety models by mainly pointing out the presence of increased lipid peroxidation, protein carboxylation, and protein thiol oxidation and decreased vitamin E levels (Desrumaux et al. 2005).

Furthermore, other evidences of oxidative stress in an anxiety rat model of social stress were demonstrated by an important increase of plasma 8-isoprostane and hippocampus protein carbonylation, but interestingly without any changes in prefrontal cortex and amygdala regions (Patki et al. 2013). Also, decreased antioxidant enzyme rates of Mn SOD and Cu/Zn SOD in the hippocampus were found in a modified model of resident-intruder paradigm to highlight social stress (e.g., social defeat model) (Solanki et al. 2015).

In addition, employing a model of PTSD induced by a single prolonged stress, it was noticed that the decreased levels of glutathione reductase found in the amygdala significantly elevated when grape powder treatment was applied. Also, when grape powder was given before the inducing of PTSD model, it was observed that raises in oxidative rates and inflammation were prevented, as proven, for example, by the analysis of plasma 8-isoprostane levels (Ghio et al. 2011).

In addition, by using a chronic social isolation model which is designed to induce depressive and anxiety-like behavior in rats, some other authors studied the effect on the hepatic oxidative stress and inflammation levels for olanzapine, an atypical antipsychotic that is also used sometimes as an adjuvant in anxiety or depressive states of bipolar disorder (Todorovi´c et al. 2016). In this way, they saw that although the drug was able to reverse decreased hepatic glutathione levels, it did not alter the elevated hepatic proinflammatory cytokines, possibly indicating that it might have favorable antioxidant proprieties, but no effect on inflammation (Jeding et al. 1995).

In fact, it has been observed after studying the effects of treatments with typical and atypical antipsychotics that while the first ones presented mainly prooxidant effects (Parikh et al. 2003, Pillai et al. 2007, Wang et al. 2005), the latter has proven to have an important antioxidant capacity (Xu et al. 2008). Also, our group previously showed some important antioxidant modifications for some atypical antipsychotics such as quetiapine, olanzapine, and risperidone (Padurariu et al. 2010).

Actually, we also demonstrated the relevance of some animal studies affective manifestations, especially on anxiety related behavior in the elevated plus maze specific test and the correlation of its factors (e.g., time in open arms, head dipping, and stretching behavior) with the main markers of the oxidative stress from the amygdala (e.g., SOD, GPX, or MDA), as a result of angiotensin or angiotensin II blockers administration, which resulted in anxiolytic

effects (Bild et al. 2013, Vignes et al .2006). In addition, a restoration for the lipid peroxidation processes and nitrite concentration was obtained after coadministration of melatonin and buspirone in a specific immobilization stress test known to induce anxiety-like behavior (Kumar et al. 2014).

Also, other antioxidant and anxiolytic agents have been proven to be effective against the oxidative stress status such as epigallocatechin gallate (EGCG), green tea polyphenol (Kolosova et al. 2006) and chlorogenic acid, a dietary polyphenol (Post, 2015) as we will insist in the last chapter of this minireview dedicated to the relevance of the antioxidant administration in the affective disorders.

Therefore, oxidative stress metabolism appears to have important implications in the evolution of replicated affective disorders aspects in animal models, but there is not yet a clear explanation to why these processes occur. Consequently, the need to further develop animal models and strategies is highlighted that will eventually lead to an elucidation of the oxidative stress mechanism in the affective disorders. Also, in the last chapter of this work we will also describe an animal model of anxiety, based on serotonin manipulation – chapter III.3.3.

### b. Genetic aspects in the understanding of oxidative stress implications in affective disorders

Analyzing the genetic profile in the most common psychiatric could help to find an explanation for the high prevalence of several diseases, the paradigm of genetic inheritance has been discussed. In this way, it seems that several classical diseases such as high blood pressure, diabetes, and also some neurodegenerative disorders may be able to pass between generations (Craddock et al. 2006). In fact, it is now well accepted that a strong inheritable genetic component might be involved in the pathogenesis of the affective disorders (McGuffin et al. 2003). In this way, many genes have been described to be involved in affective disorders' pathology. They may be associated with brain growth factors, signal molecules, receptors, chelation, and transport factors, while some of them are actually genes which encode several enzymes and factor implicated in brain oxidative status (McGuffin et al. 2003).

Although the way in which the genetic component actually increases the risk for affective disorders is not fully understood, it is believed that the genetic components of affective disorders may be a result of multiple gene modifications that lead to a specific environmental factor-dependent liability. In this way, the bipolar disorder was found to be the most likely to be inherited (up to 80% probability by additive genetic factors) (Kindler et al. 1993) while major depression (40%– 70%) (Eley et al. 2002) and anxiety (40–50%) (Norrholm et al. 2009) are less probable to be inherited by the descendants.

We should keep in mind that a contradiction between theoretical heritability and susceptibility premises and the actual clinical status may occur. A good example in this way is the genetic component of PTSD, which seems to occur in particular genetic context and under particular environmental factor (Skelton et al. 2012). Moreover, a significant interaction between three polymorphisms in the GABA receptor gene was reported to be involved in PTSD prediction in correlation to childhood trauma severity (Galanopoulou et al. 2008).

Besides the neurotransmission regulatory function of GABA receptors, it seems that GABAalpha-2 receptor is also implicated in stress modulation via chloride cotransporters

domains, which are also activated by oxidative stress responsive kinases (Zmijewski et al. 2001). In this way, since oxidative stress may be a molecular response to psychological stress, it might actually modulate the regulator of G-protein signaling 2 (RGS2) (Nunn et al. 2010) which is a part of the adrenergic receptors during conditioned fear response. Also, recently several single nucleotide polymorphisms of the FK506 binding protein 5 (FKBP5) were found to interact with childhood trauma in order to create PTSD susceptibility (Binder et al. 2008).

In a similar way, oxidative stress may be involved in FKBP5 functionality due to the interaction between FOXO1 (a transcription factor involved in cell survival and modulated by oxidative stress), glucocorticoid receptors, and increased levels of psychological stress (Guidotti et al. 2013). In addition, the female predisposition to PTSD may be modulated by a recently found single nucleotide polymorphism of estrogen response element, found on pituitary adenylate-cyclase 1 receptor gene (Ressler et al. 2011). It seems that pituitary adenylate-cyclase activating peptide/pituitary adenylate-cyclase 1 pathway exhibits a role in psychological stress response, which is dependent on an estrogen response element that conveys sex specific-modulation of fear response. Moreover, this pathway seems to be involved also in an oxidative stress protective system against ROS-induced mitochondrial dysfunctions and apoptosis (Masmoudi-Kouki et al. 2011).

Also, dopamine and serotonin receptors polymorphisms may also be involved in PTSD predisposition due to the limbic-frontal neurocircuitry complexity. In this way, dopamine transporter SLC6A3 and promoter region of the serotonin transporter genes polymorphisms seem to give high risk of PTSD, especially in increased risk environment factors (Galanopoulou et al. 2008). Dopamine receptor D2 association with oxidative stress is rather controversial, considering, for example, that one recent study correlated a dopamine D2 receptor antagonist and anti-Parkinson medication with reduced excitotoxicity and therefore reduced neuronal apoptosis in oxidative stress conditions (Odaka et al. 2014).

Moreover, another previous study also associated D2 and D3 dopamine receptor agonists with glutamate oxidative stress inhibition in oxygen/glucose deprivation models (Rosin et al. 2005). In addition, other groups correlated organophosphates exposure with oxidative stress and alterations in brain dopamine and serotonin receptors of young rats, but still no actual correlation between oxidative stress and these receptors has been proposed (Sankhwar et al. 2016).

In fact, although a clear correlation between genetic components and PTSD has been made and all of these genes may be directly or indirectly implicated in oxidative stress modulation or development, no previous correlation between all of them is available. Therefore, since the predisposition to PTSD through these genes polymorphisms has been shown, the oxidative stress pathways in which they may be involved are almost unknown in PTSD conditions. In this way, it can be stated that the genetic component, the environmental risk factors, and their interaction in the affective disorders development context are rather variable.

Based on this observation, the latest studies in PTSD genetics actually revealed that identifying the specific genes or neurobiological pathways involved in PTSD development and the specific modifiable environments associated with PTSD risk (as well as the mechanism of interaction between the two) could broaden posttrauma intervention approaches in PTSD therapy or even result in some prevention mechanism (Skelton et al. 2012). Modern molecular biology and developmental biology rely on a crucial paradigm.

As all living organisms are the result of a complex interaction between genome and environment, the mental disorders seem not to deviate from this pattern. In this way, interesting questions could arise: in what way the genetic component would formulate a sufficient background for affective disorders pathological development and, on the other way around, how complex would the environmental interaction be in order to provide sufficient risk for pathologies to occur via oxidative stress development?

In this way, it seems that both questions eventually got answers that it has been shown by familial studies (Low et al. 2008, Merikangas et al. 2009) that several genetic modifications (mutations or polymorphisms) in key genes could give rise to a sufficient mood imbalance background. Unfortunately, as the familial cases are thought to be easier to screen and to prevent, these are only 5 to 10% of all cases. On the other hand, twin and adoption studies (Smoller et al. 2003, Na et al. 2011) revealed that a close interaction exists between the genetic background and the environment, raising several environmental risk factors which could play an important in building up the risk for affective disorders development. There are plenty of studies that revealed the genetic component implications in the affective disorders occurrence and rarely the same susceptibility locus shows up repeatedly.

This is the reason why a genetic screening in affective disorders is hard to produce any preventive actions. In this way, a variety of genes have been shown to be involved in affective disorders development (reviewed by McGuffin et al. 2003). Association analyses of panic genetics showed several classical candidates such as monoamine oxidase A (MAO-A), catechol-O-methyl transferase (COMT), adenosine A2A receptor and cholecystokinin B receptor genes [205]. COMT gene, for example, codes for a catecholamine catabolic breakdown enzyme, which is known to be involved in anxiety development as high levels of COMT have been observed in patient's serum (Lehman et al. 2002).

The implication of the most common COMT polymorphism in panic disorder is also rather controversial due to extremely different study results (reviewed by Na et al. 2011). In this way, although there are studies which negatively correlate COMT with PD, it seems that this polymorphism remains as one of the most consistent findings in genetics of panic disorder.

Furthermore, the correlation between COMT and oxidative stress has not been studied much. Still there are several studies which report high COMT activity and high oxidative stress levels in vitiligo patients (Mehaney et al. 2014, Colucci et al. 2015), a more relevant correlation between genetic implications in PD and oxidative stress may be made regarding the mitochondrial monoamine oxidase.

Also, MAO has been correlated with panic disorder due to several polymorphisms which modulate MAO gene transcriptional activity (J¨onsson et al. 2003). Furthermore, a gender specific modulation has been demonstrated and was associated with PD in several populations (Deckert et al. 1999). The association remains controversial due to the fact that in other populations this correlation failed to be shown (Hamilton et al. 2000). Also, the cholecystokinin (CCK) neuropeptide has been associated with PD development. It seems that an interaction between CCK and dopamine may be involved in panic attacks modulation (Vanderhaeghen et al. 1975).

In fact, ambiguous results have been obtained through time in several studies and therefore the exact correlation between CCK and PD is not known, although it seems that CCK

A receptor and dopamine D5 genes are closely situated on the short arm of the fourth chromosome (Vanderhaeghen et al. 1975).

Another interesting association is referring to connection between the serotonin transporter gene and both panic disorder (Kuppi et al. 1995) and OCD (Strug et al. 2010) pathologies. This gene is coding for a protein affected by selective serotonin reuptake inhibitor (SSRI) medications, which are of course frequently used in anxiety disorders treatment (Wendland et al. 2008).

Interestingly enough, discrimination was made by showing that the modifications that lead to panic or OCD are different and a question arises: why the way in which a molecule is modified can change the pathological features? Maybe the answer relies on the genetic components involved in these two diseases development. In this way, the same gene may possess different polymorphic alleles of which different or opposed interactions lead to different results. For instance, the serotonin receptor 2A was associated with both PTSD and panic disorder (Ravindran et al. 2009).

Regarding the bipolar disorder genetic component, it seems that most of the genetic studies on this matter focused on the neurotransmitter systems, which can be influenced by medication, and particularly dopamine, serotonin, and noradrenalin systems. In this way, direct implications were demonstrated for the monoamineoxidase A, 5-hydroxytryptamine transporter, and catechol- methyl transferase genes (Maron et al. 2005, Preisig et al. 2000). Also, the implications of these molecules in oxidative stress status have been partially explained, but no direct correlative study has been carried out.

Moreover, Menazza et al. (2010) showed that MAO A activity may increase mitochondrial ROS production, which will lead to increased oxidative stress and myofiber damage. In this way, increased MAOA activity in brain tissue may also lead to increased oxidative stress, knowing that the neurons are mitochondria-rich high energy consumers. In the same way, COMT activity is thought to be oxidative stress promoter in association with high catalase activity in melanocytes and melanin biosynthesis (Colucci et al. 2015).

Also, while COMT plays an important role in the brain catechol amines degradation, it also degrades dopamine in the prefrontal cortex area which leads to working memory correlated tasks resolving. Since impaired working memory has also been correlated to oxidative stress and damage (Bild et al. 2013) it might be possible that high COMT activity is associated with both agitation/disorientation and oxidative stress.

Later, D-amino acid oxidase activator gene and brain derived neurotrophic factor gene became also of great interest, but it seems that no actual evidence was found in this matter (Green et al. 2003, Hattori et al. 2003) mainly due to the fact that most of these genes are reported as schizophrenia susceptibility genes too. Surprisingly, it can be observed that both D-amino acid oxidase activator and brain derived neurotrophic factor are involved in several oxidative stress pathways (Cabungcal et al. 2013) but a direct correlation between these two and oxidative stress in bipolar disorder has not been yet showed.

In the same way, several susceptibility genes were shown in depression and anxiety development. In addition, the brain derived neurotrophic factor (BDNF) polymorphism Val66Met thought to be implicated in bipolar disorder was also investigated in depression. Just as in other genes' case, the results were quite controversial.

In this way, no significant association with BDNF polymorphism or inconsistent evidence was reported (Schumacher et al. 2005, Surtees et al. 2007). In spite of these reports, other variations in the BDNF gene may be influencing the susceptibility to depression disorder (Hashimoto, 2010). Thus, recently a link between BDNF and oxidative stress has been confirmed in schizophrenia (Zhang et al. 2015). It seems that the patients exhibit a significant decrease in BDNF levels and also in the activities of SOD and GPX.

Moreover, Numakawa et al. (2014) showed that significant correlations can be made regarding BDNF and SOD specific activity. Also, they suggested that an interaction between BDNF and CAT could be associated with PANSS scale as a cognitive factor. Furthermore, a similar PANSS factor (PANSS depressive factor) can be correlated with the interaction between BDNF and GPX. In this way, a possible association between BDNF and inflammatory cytokines and also hypothalamic-pituitary-adrenal (HPA) axis could emerge.

Another gene thought to be implicated in depression is tryptophan hydroxylase gene, which encodes for an important rate limiting enzyme of brain serotonin synthesis (Lohoff, 2010). It seems also that a specific brain isoform of tryptophan hydroxylase (TPH2) may be the connection between serotonergic systems and depression and bipolar disorder (Zill et al. 2004). In this way, both Zill et al. and Zhang et al. (2005) groups reported genetic modifications that could link TPH2 gene to depression susceptibility.

Furthermore, Kuhn et al. (2011) reported a possible implication of the oxidative stress status in TPH2 activity, whereas THP2 oxidation leads to low TPH2 enzymatic activity. In addition, it seems that 5-hydroxytryptamine (5HT) synthesis by miss folding and aggregation due to the cysteine-rich structure could also be highly susceptible to oxidative damage (Weng et al. 2016).

Furthermore, a very recent report showed increased systemic oxidative stress in TPH2 knock-out mice and also increased lipid metabolism impairments which might be implicated in serotonin deficiency (Weng et al. 2016). Other genes were similarly correlated to the depression development, especially considering the wide implication in the oxidative stress status and the association between high oxidative stress and depressive symptoms. In this way, it was reported that a polymorphic variant glutamic acid decarboxylase 2 that is described as an enzyme involved in GABA synthesis and which seems to be severely impaired in anxiety disorders (Lydiard, 2003).

It also seems that GAD2 enzyme is involved in an extensive antioxidant system yielded by the astrocytes. In this way, several reports showed that enhanced GAD2 activity may contribute to neuronal protection from oxidative stress in vitro neuronal tissue cultures (Lamigeon et al. 2003). Also, controversial results were obtained in the case of the polymorphisms for the regulator of G-protein signaling 2 (RGS2) gene, which could be implicated in anxiety-like behaviors (Leygraf et al. 2006) but also in PTSD, emotional distress, and panic disorder (Koenen et al. 2009).

In fact, a correlation between RGS2 and oxidative stress was made in a report regarding the postischemic RGS2 upregulation, which leads to enhanced apoptosis in astrocytes via oxygen-glucose deprivation (Endale et a. 2010). Similarly, another RGS family protein, called RGS4, has been correlated with oxidative stress in postischemic and neurodegenerative disorders. In this way, it seems that a common lipid peroxidation product such as 4-hydroxy-2-nonenal can inhibit RGS4, which further impairs the GTPase activity (Monroy et al. 2013).

Several single nucleotide polymorphisms (SNPs) within the transcriptional coactivator PPARGC1A were also associated with the anxiety phenotypes. PPARGC1A was actually discovered in the muscle cells and brown fat and thought to stimulate mitochondrial biogenesis by increasing oxidative phosphorylation and by enhancing oxidative respiration (Wu et al. 1999), but it has been shortly connected to the nuclear respiratory factors 1 (NRF1) and 2 (NRF2), which are linked to oxidative stress and also well correlated to anxiety both in human and rodent models (Distler et al. 2012).

However, we have to mention that a strong correlation between genetics, oxidative stress, and affective disorders has not been made quite clear due to the extreme genetic variability of the individuals. Interestingly enough, many correlations were observed between these susceptibility loci and oxidative stress status observed in affective disorders. Also, as mentioned before, some of the genes code for proteins which are important enzymes involved in oxidative or phosphorylation reactions which commonly use ROS.

In this way, at least hypothetically and theoretically, a link between the neuroprogression biomarkers and the decline observed in affective disorders can be made. Also, due to the complex mechanisms underlying the pathophysiology of these diseases, the way in which inflammatory processes, oxidative stress, mitochondrial dysfunctions, and apoptosis are interacting is rather problematic and controversial.

### c. Antioxidant approaches for the affective disorders treatment

Of course, the reason behind studying the connection between the affective disorders and the oxidative stress status is represented by the need of exploring new approaches towards the management of these important psychiatric disorders. This acute need of finding new therapeutic methods is sustained by the position these disorders are occupying on a worldwide scale. In this way, according to an estimative perspective given by the World Health Organization (WHO), major depressive disorder will be the second health problem worldwide by the year of 2020 (WHO, 2008). Even more, it seems that in Europe and USA anxiety disorders are the most prevalent psychiatric conditions (Nutt et al. 2007, Kessler, 2007).

In this way, by knowing that oxidative stress is an important component in many diseases, the idea of counteracting its effects emerged lately in the literature. Thus, several approaches were designed, such as oxidant potential inhibition and antioxidant system potentiation approaches.

Therefore, several studies showed that oxidative stress in the affective disorders can also be inhibited by psychiatric disease therapy itself, as this was demonstrated to be a causative component (Bakunina et al. 2015, Sousa et al. 2014). For instance, lithium can exhibit mood stabilizing properties, but also antioxidant potential, as some early phase studies regarding the oxidative stress status in bipolar disorder showed (Kiełczykowska et al. 2006). Thus, a six-week lithium therapy can successfully decrease lipid peroxidation markers and restore SOD plasma levels (Machado-Vieira et al. 2007).

Moreover, these results were comparable to prior studies on BD patients after manic and euthymic episodes (Banerjee et al. 2014). Furthermore, Banerjee et al. (2014) group showed that lithium antioxidant activity is linked to Na+-K+-ATPase activity. Also, when the modification of oxidative stress markers was followed in patients with depressive disorder after

fluoxetine administration for three months, it was found that the levels of some antioxidant enzymes like Cu/Zn-SOD and CAT are significantly higher in the patients group versus the controls (Gałecki et al. 2009).

Moreover, it seems that also MDA levels are significantly lower during MDD medication (Atmaca et al. 2004). Additionally, it seems that oxidative unbalance in social phobia patients can be corrected after eight weeks of citalopram administration (Frey et al. 2007).However, further details on the antioxidant matter in the psychiatric disorders will be described in the future chapter, referring to the future projects in the academic, professional and research field.

Regarding the latest developments on the antioxidant approaches in the broad spectrum of the affective disorders, we can mention also the studies on the antioxidant extracts, such as the study from 2019 regarding the antioxidant capacity and behavioral relevance of a polyphenolic extract of Chrysanthellum americanum in a rat model of irritable bowel syndrome (Cojocariu et al. 2019).

In fact, lately there is an increased interest in the context of the neurovisceral disorders impairing the brain-gut axis, which could partially explain the neurosomatic features of disorders such as the one mentioned above (the irritable bowel syndrome-IBS, which is one of the most important and current functional gastrointestinal disorders that can be diagnosed only based on symptomatological criteria) (Padhy et al. 2019).

In fact, regarding these neurological-related implications, it was previously and bi-directionally showed that depression, anxiety, ataxia, and attention deficit hyperactivity disorder could exhibit strong gastrointestinal manifestations, while neuropsychiatric disorders such as schizophrenia, autism, peripheral neuropathy are usually including also IBS-like features in their symptomatology (Fadgyas Stanculete et al. 2014).

In this way, it seems that all these three factors (oxidative stress, inflammation and neurological (Balmus et al. 2020). For example, Lakhan and Kirchgessner et al. described a possible pathway of neuroinflammation involved in enteric nerve system impairment that would lead to hyper excitability followed by impaired intestinal motility. Thus, immune cells such as mast cells or enterochromatoffin cells tend to overreact in the colonic mucosa signalling inflammation to the enteric nervous system, which could further trigger serotonin and several cytokines mediation into local reactive oxygen production (Lakhan et al. 2020).

**d. Focusing on serotonin manipulation on the anxiety and affective disorders modeling**

As mentioned above, the dysregulation of both hypothalamic–pituitary–adrenal (HPA) axis and serotonergic system have been implicated in the pathophysiology of several disease states associated with the response to stressful stimuli, such as anxiety and different affective disorders (Heisler et al.2003, 2007). Generally, reducing serotonin (5-hydroxytryptamine, 5-HT) levels or activity increases the responsiveness to stress. As a result, the HPA axis becomes hyperactive, behavioral responses are accentuated, and animals may overreact to demanding situations ( Temel et al.2003). It has been demonstrated that the activity of tryptophan hydroxylase, the rate-limiting enzyme for 5-HT production, is increased by sound stress (Chung

et al. 1999) or that the development of learned helplessness in rats after inescapable stress is related to the depletion of 5-HT in the prefrontal cortex (Petty et al. 1992).

Additionally, depletion of 5-HT by 5,7-dihydroxytryptamine (5,7-DHT) neurotoxin reduced anxiety in rats as tested by behavior in elevated plus maze (Briley et al. 1990) and increased impulsive responses (Harrison et al. 1997). Also, studies with experimental animals have provided evidence tha serotonergic system is involved in cognitive process, but its role in cognition is rather controversial ( Hritcu et al.2007, Lieben et al. 2006, Cassaday et al. 2003). Activity of the corticotropin-releasing hormone (CRH) neurons in the hypothalamic paraventricular nucleus (PVN) forms the basis of the activity of the HPA-axis. Previous studies indicated that PVN ablation dampens stress-induced CRH secretion (Caldeira et al. 2000, Kiss et al. 2001). It has been demonstrated that when CRH antagonists were centrally infused in rats submitted to aversive stimulation, responses such as freezing or withdrawal were attenuated or blocked ( Bennett et al. 2008, Holsboer et al. 2008, et al. 2007).

It is believed that 5-HT stimulates the HPA axis via action at CRH-containing neurons in the brain, which subsequently augments glucocorticoids release. PVN is also strongly implicated in the redox mechanisms and in increasing reactive oxygen species (ROS)-producing machinery ( Han et al. 2005, Guggilam et al. 2007, Kang et al. 2008). In addition, several studies showed that the neurotoxic effects of 5,7-dihydroxytryptamine may involve oxidative stress generation ( Del Rio et al. 2002, Gibb et al. 1990). The current study was therefore designed to determine whether a unilateral 5,7-DHT lesion of the parvocellular PVN neurons, leads to a deactivation of HPA-axis and affects the anxiety state and cognitive functions.

Methodologically, we did that by selecting male Wistar rats, weighing approximately 200-250 g at the beginning of the experiment. The animals were housed in a temperature- and light-controlled room (22°C, a 12-h cycle starting at 08:00 h) and were fed and allowed to drink water ad libitum. Rats were treated in accordance with the guidelines of animal bioethics from the Act on Animal Experimentation and Animal Health and Welfare Act from Romania and all procedures were in compliance with the European Communities Council.

Thus, the unilateral lesion was preferred in order to avoid the debilitating consequences of a bilateral lesion or other adverse effects. We used in our studies two different doses of 5,7-DHT: 8µg/3µl and 16µg/3µl. The injection volume was established in accordance with previous studies (Feldman et al. 1998, Weidenfeld et al. 2002). Sham-operated and lesioned rats were subjected to a battery of behavioral tests designed to assess anxiety behavior through the elevated plus maze task and spatial memory formation through the radial arm maze task.

Further, we were interested to know whether this 5,7-dihydroxytryptamine lesion of the PVN would result in an imbalance in oxidative stress levels of temporal brain area, the most vulnerable cortical area to oxidative stress effects (Karelson et al. 2001) by measuring the extent of some lipid peroxidation products like malondialdehyde (MDA) and defense enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPX).

Also, we did search for possible correlations between the behavioral response to this lesion and brain oxidative stress. We also did a histological determination, since in the majority of PVN– lesioned rats (30/36) the point of the syringe needle was positioned in the parvocellular part of the PVN. Thus, regarding the effects of 5,7-DHT induced lesion of PVN on elevated

plus maze behavior, which is mainly used to assess exploration and anxiety status, in our experiment, we used 2 different doses of 5,7-DHT: 8 µg/3 µl and 16 µg/3 µl.

The lesioned rats spent significantly more time in the open arms in both lower ($F(1,28)=156$, $p= 0.0005$) and higher dose ($F(1,28)=33$, $p= 0.0003$) of 5,7-DHT, compared to sham operated rats, suggesting that the lesion of PVN significantly diminished anxiety-like behavior (Figure 11). Also, post hoc analysis revealed significantly statistical differences between 5,7-DHT (8 µg/3µl) and 5,7-DHT (16 µg/3µl) groups ($p=0.0009$). These results support the hypothesis that 5,7-DHT lesion of PVN affected the processes associated with initiation or maintenance of behavioral responses to novel and/or exploration situations.

Regarding the effects of 5,7-DHT induced lesion of PVN on spatial memory in radial 8-arm maze task, the lesion of the PVN parvocellular neurons with 5,7-DHT significantly impaired short-term memory in both lower ($F(1,18)=12$, $p= 0.0026$) and higher ($F(1,18)=13$, $p=0.0021$) dose, expressed by the number of working memory errors during 10 days of testing in radial arm-maze task (Figure 4.3).

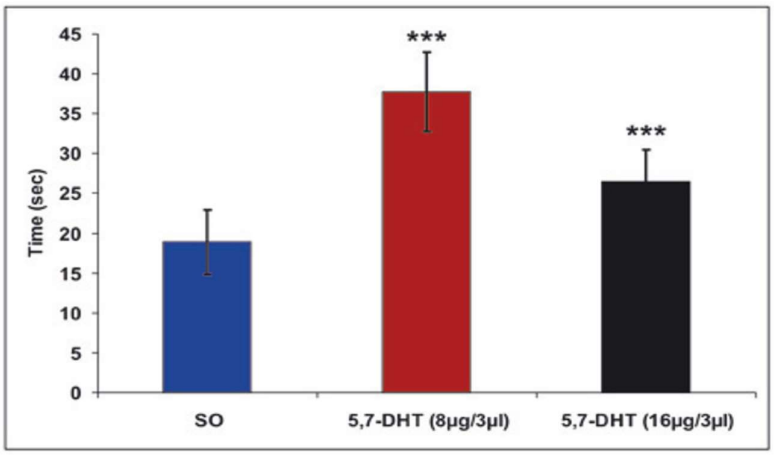


Figure 4.3. Effect of 5,7 DHT (5,7-dihydroxytryptamine) induced hypothalamic paraventricular nucleus lesion (8µg/3µl, 16µg/3µl) on the time spent in the open arms of the elevated plus maze. The values are mean ± S.E.M. (n=15 animals per group).*** $p<0.001$ vs. sham-operated (SO) group.

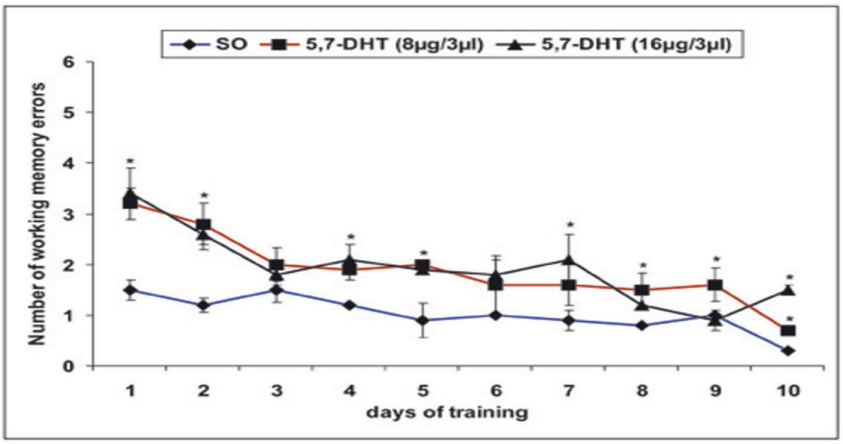


Figure 4.4. The number of working memory errors in the 5,7 DHT (5,7-dihydroxytryptamine) lesioned rats (8µg/3µl, 16µg/3µl) during 10 days of training. The values are mean ± S.E.M. (n=15animals per group). *$p<0.05$ vs. sham-operated (SO) group.

Post hoc analysis showed no difference between 5,7-DHT (8µg/3µl) and 5,7-DHT (16 µg/3µl) groups (p=0.39). 5,7-DHT induced lesion of the PVN did not result in a significant change of reference memory in 5,7-DHT (8µg/3µl) (F(1,18)=0.0009, p= 0.9) and 5,7-DHT (16 µg/3µl) (F(1,18)=0.0008, p= 0.9) groups, as expressed by the number of reference memory errors during 10 days of testing in radial arm-maze task (Figure 4.4).

Post hoc analysis revealed no significant statistical differences between 5,7-DHT (8 µg/3µl) and 5,7-DHT (16 µg/3µl) groups (p=0.999). Also, the time taken to consume all five baits was significantly decreased in lower (F(1,18)=5, p= 0.03), but not in the higher (F(1,18)=3, p= 0.1) dose of 5,7-DHT, compared to sham-operated group (Figure 4.5). Additionally, post hoc analysis revealed no significant statistical differences between groups (p=0.101).

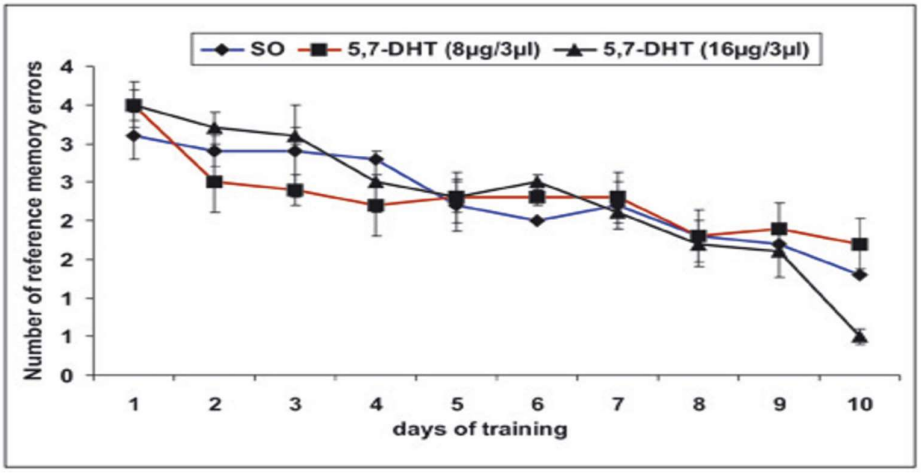


Figure 4.5. Number of reference memory errors in the 5,7 DHT (5,7-dihydroxytryptamine) lesioned rats (8µg/3µl, 16µg/3µl) during 10 days of training. The values are mean ± S.E.M. (n=15 animals per group).

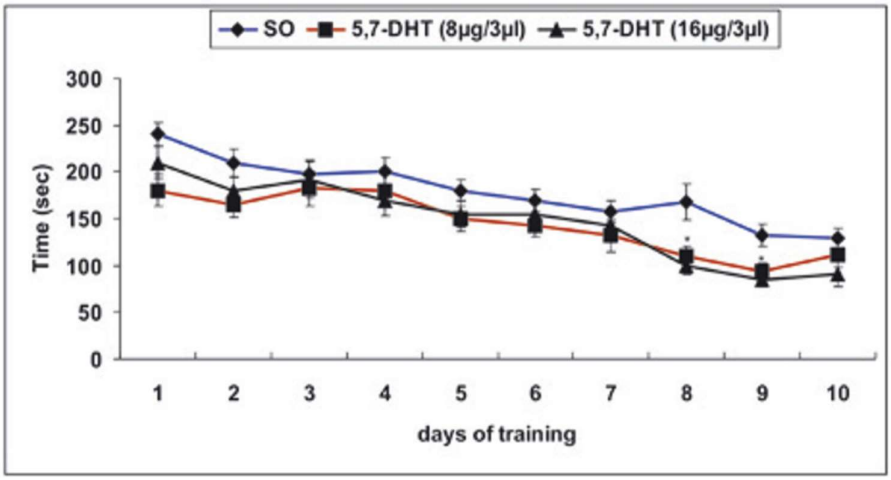


Figure 4.6. The time taken to consume all five baits during 10 days of training in the 5,7 DHT (5,7-dihydroxytryptamine) lesioned rats (8µg/3µl, 16µg/3µl). The values are mean ± S.E.M. (n=15 animals per group). *p<0.05 vs. sham-operated (SO) group.

Also, when we determined the linear regression between the number of working memory errors vs. oxidative stress markers (SOD, GPX and MDA), we only found significant positive correlations in the case of working memory errors vs. MDA (n=30, r =0.536, p=0.002) (Figure 4.6), while working memory errors vs. SOD (n=30, r =0.271, p=0.147) and working memory errors vs. GPX (n=30, r =0.152, p=0.422) resulted in no significant correlations. These data suggest that the increase number of working memory errors in 5,7-DHT PVN-lesioned rats, could be correlated with the involvement of 5,7-DHT in oxidative stress generation.

Regarding the oxidative stress status results we did obtained, the 5,7-DHT lesion of PVN resulted in no significant modification of SOD specific activity in both 8 µg/3µl ($F(1,28)=3$, $p= 0.07$) and 16 µg/3µl ($F(1,28)=1$, $p= 0.33$) doses, compared to sham operated group. Post hoc analysis also revealed that the groups did not differ from each other ($p= 0.487$). In addition, no significant modifications of GPX activity were observed in 8 µg/3µl ($F(1,28)=1$, $p= 0.2$) or 16 µg/3µl ($F(1,28)=3$, $p= 0.09$) groups, compared to sham operated rats.

However, post hoc analysis showed a significant difference between 5,7-DHT (8 µg/3µl) and 5,7-DHT (16 µg/3µl) groups ($p= 0.011$). The MDA level from the temporal lobes homogenates was significantly increased in 5,7-DHT (8µg/3µl) ($F(1,28)=125$, $p=0.0007$) and 5,7-DHT (16µg/3µl) ($F(1,28)= 49$, $p=0.0001$) groups, compared to sham-operated rats, suggesting pro-oxidant effects. In this case, post hoc analysis revealed no significant differences between 8 µg/3µl and 16 µg/3µl groups ($p= 0.52$) (Table 10).

Table 10. The effects of 5,7-dihydroxytryptamine induced lesion of hypothalamic paraventricular nucleus on SOD and GPX specific activities and on MDA level. a- Each value represents mean and standard deviation. ***p<0.001 (one-way ANOVA).

| | Sham-operated[a] (n=15) | 5,7-DHT[a] (8µg/3µl) (n=15) | 5,7-DHT[a] (16µg/3µl) (n=15) |
|---|---|---|---|
| SOD (U/mg protein) | 0,809 ± 0,04 | 0,853 ± 0,03 | 0,888 ± 0,24 |
| GPX (U/mg protein) | 4,146 ± 0,6 | 4,026 ± 0,35 | 4,747 ± 0,78 |
| MDA (nM/mg protein) | 14,87 ± 2,12 | 44,24 ± 12,55*** | 43,46 ± 16,8*** |

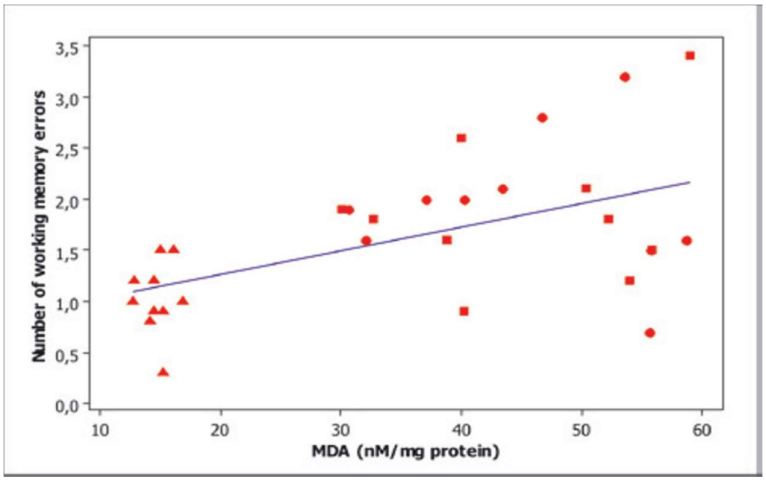


Figure 4.7. The correlations between working memory errors vs. malondialdehyde, in sham-operated (SO)(▲), 5,7-DHT (8µg/3µl) (●) and 5,7-DHT (16µg/3µl) (■) rats.

Thus, the results provided above are showing additional evidence that the lesion of thePVN with 5,7-DHT results in changes of the mechanisms mediating behavioral responses to novel situations, and contribute to studies that have begun to define neural interactions that are essential for integrated stress responses.

Moreover, we showed a direct correlation between the lipid peroxidation processes and the aforementioned behavioral changes.

In the present experiments, 5,7-DHT-lesioned rats lacking parvocellular PVN neurons display patterns of behavior generally consistent with a diminution of fearfulness in novel situations. Consideration of the known physiological roles for parvocellular PVN neurons and our behavioral data lead us to hypothesize that elimination of these neurons may effectively reduce autonomic responses to environmental stimuli.

The end result of such neurohormonal impairment may be translated into a reduction in fear-induced behaviors associated with unfamiliar properties of the environment and perhaps a general decrease in the efficiency which information is processed (via changes in "arousal" level).

Our results above also support the hypothesis that endogenous parvocellular PVN neurons are involved in physiological processes mediating endocrine and behavioral responses to exogenous stimuli, serving to activate the animal and promote adaptation to environmental change. In this way, destruction of the parvocellular PVN, a major source of brainstem and median eminence-projecting neurons, results in behavioral changes consistent with reductions in magnitude of situation specific fear responses, which are associated with activation of these stress systems (Oldfield et al. 2001).

Also, in our experiments, the patterns of behavior observed in rats with 5,7-DHT lesions of the PVN are suggestive for an alteration in arousal level. Lesions of the parvocellular PVN induced an increased in time spent in the open arms of the elevated plus maze task, trend which has been negatively correlated with behavioral and physiological indices of "fear" in rats ( Fabricius et al. 2008, Ohmura et al. 2008).

The differences observed between 5,7-DHT lesioned rats and sham-operated rats in elevated plus maze task suggest that lesions of the PVN attenuated cautious behavior. This alteration in emotionality and arousal level was also reported by other authors that demonstrated a heightened incidence of central and peripheral ambulation and increased rearing in the open field test, in rats with ibotenic acid-induced lesion of the PVN ( Herman et al. 1990).

Moreover, Briley demonstrated an increased ratio of open/total arm entries in the elevated plusmaze, as a result of intracerebroventricularly administration of 5,7-DHT, reflecting a decreased level of "anxiety" ( Briley et al.1990).

Additionally, 5,7-DHT induced an impairment of short-term memory, as shown by the increased number of working memory errors, in both in 5,7-DHT (8µg/3µl) and 5,7-DHT (16µg/3µl) groups, compared to sham-operated group, without affecting long-term memory, assessed by the number of reference memory errors in the radial arm maze task.

Regarding the possible relationship between the aforementioned changes in anxiety state and the impairment in working memory, it can be speculated that since reference memory is unimpaired, the working memory deficit could be the result of the same underlying process related to arousal level.

In this way, Sandstrom demonstrated in 2005 that isolation stress, during the third postnatal week in male rats, increase plasma corticosterone level and results in impairments of working memory but not reference memory erros, in a 12-arm radial maze task (Sandstrom et al. 2005).

The involvement of PVN in memory consolidation is still not fully understood. Some studies demonstrated that PVN lesion did not alter retention of shock avoidance and extinction of the learned avoidance response, but impaired performance of both the delayed non-match to sample paradigm from T-maze and sensory discrimination tasks, suggesting impairment in ability to learn the discriminative cues required for accurate choice behavior under the respective conditions (Herman et al.1990).

In another study by Aisa et al. it was demonstrated that rats exposed to stressful stimuli (e.g. maternal separation) showed elevated levels of CRH mARN in the PVN and significant learning impairments both in the Morris water maze and in the novel object recognition test (Aisa et al. 2007).

Also, previous studies suggested a controversial role of serotonergic systems in cognitive processes. Serotonergic lesions have been reported to have no effect (Hagan et al.1990), facilitate (van der Staay et al. 1999) or impair (Hritcu et al. 2007) performance in various learning tasks.

In addition, it has been demonstrated that 5,7-DHT lesion of the dorsal raphe nuclei affects object recognition task, but does not impair affective behavior and corticosterone response to stressor (Lieben et al. 2006). Other authors have reported that intracerebroventricular 5,7-DHT lesion disrupts acquisition of a working memory task or the place navigation task in watermaze (Cassaday et al. 2003).

Moreover, we were interested in the influence of 5,7 DHT induced lesion in the PVN on neuronal oxidative stress status. Some other studies confirmed that PVN is extremely important in the redox mechanisms and increasing ROS-producing machinery in angiotensin II-induced sympatho-excitation, hypertension or congestive heart failure, via cytokines signaling that may involve a modulation of catecholamine levels (Han et al. 2005, Guggilam et al. 2007, Kang et al. 2008). Also, there are some reports that suggest a possible role of oxidative stress mechanisms in the 5,7-DHT induced neurotoxicity ( Del Rio et al. 2002, Gibb et al.1990).

As in the case of 6-OHDA, another commonly used tool to investigate the mapping of neuronal pathways, the toxicity of 5,7-DHT can be attributed to its oxidation by molecular oxygen and/or monoamine oxidase into quinones and the production of the by-product hydrogen peroxide ($H_2O_2$), which can be transformed into highly reactive hydroxyl radicals and produce cell damage (Hritcu et al. 2008, Ciobica et al.2008).

This was also observed in our experiment, since the level of lipid peroxidation was increased in both 5,7-DHT (8µg/3µl) and 5,7-DHT (16µg/3µl) treated groups, as shown by the increased concentration of MDA from the temporal lobe.

Moreover, when we determined the linear regression between the numbers of working memory errors vs. MDA, we found a significant positive correlation. This could suggest that the increased number of working memory errors in 5,7-DHT treated rats, could be correlated with the involvement of 5,7-DHT in oxidative stress generation.

# SECTION II: FUTURE PROJECTS IN THE ACADEMIC, PROFESSIONAL AND RESEARCH FIELD

Our future projects will be mainly linked also to oxidative stress status/metabolism and schizophrenia, but as we detailed earlier, as a further step from what I have already did until now, on understanding how antioxidants could work on this matter.

Thus, the current pharmaceutical treatment for the tens of millions of people around the world who suffer from schizophrenia is limited to just a few antipsychotics. Despite the somewhat proven efficacy of these drugs, the overall condition resulting from treatment for schizophrenia remains below optimal levels. Thus, alternative treatment options are absolutely necessary. In this way, a possible therapeutic approach could be represented by antioxidant therapy.

As we have already described in this paper, lately, oxidative stress is attracting more and more attention. Many studies in the medical and biological fields has focused on deciphering the mechanisms underlying this process, given the growing importance of oxidative stress in multiple degenerative and mental illnesses, including schizophrenia.

Mitochondria have been extensively researched, being considered the most important source of oxidative stress, especially in terms of mitochondrial defects that are associated with a high level of oxidative stress and could decipher, at least in part, the enigmas of some diseases. Thus, the most researched, in this sense, is the complex I of the electron transport chain, from the mitochondrial level.

The body has an arsenal of protection against oxidative stress which is normally very effective. Biochemically, antioxidant agents are reducing compounds that are able to block pro-oxidant processes and therefore oxidative alterations, in redox reactions. Due to their high reactivity, free radicals have many harmful effects on the body, being involved in many pathologies, among which the most feared are cancer, atherosclerosis, chronic inflammation or diabetes. It is also known that oxidative stress has a very important role in the etiopathogenesis of several neuropsychiatric disorders, including schizophrenia, Parkinson's, Alzheimer's, anxiety or bipolar disorder, and possible in many other diseases.

Of all the tissues, the brain contains the highest percentage of unsaturated fats, so its cells are the most vulnerable to free radical attack. Also, susceptibility to free radicals is amplified by the presence at this level of an increased number of mitochondria, electron-rich neurotransmitter biomolecules as well as a high level of oxygen consumption.

There is a close link between the oxidative status of the neuron and its functionality, implicitly of the transduction pathways of biochemical signals and its degree of survival. Thus, oxidative stress exerts detrimental influences on the dopaminergic transmission system, leading to the classic changes encountered in schizophrenia. Oxidative stress may be a potent intracellular signaling mechanism that induces changes in dopaminergic type D2 receptors.

Most theories regarding the role of oxidative stress in schizophrenia refer to their effect on polyunsaturated lipids in the neuronal membrane. These aspects are also proven by analyzes indicating an increase in the concentration of phosphatidylcholine and phosphatidylethanolamine in the brain, in post-mortem analyzes in schizophrenic patients.

Because it is known that phospholipid metabolism plays a crucial role in neuronal growth and synaptic remodeling, the idea that abnormalities in these membrane components may be associated with normal neurodevelopmental disorders in schizophrenia is plausible.

There are sufficient scientific data to indicate that at least some patients with schizophrenia have low levels of PUFA and that it appears to correlate with the presence of negative symptoms. The most important PUFAs, such as arachidonic acid and docosahexaenoic acid, are important in monoaminergic neurotransmission, brain development, and functioning. This may suggest that supplementation with essential fatty acids could alleviate the symptoms of schizophrenia. It is possible that the administration of polyunsaturated fatty acids could lead to significant improvements in the clinical condition, comparable to those produced by atypical antipsychotic drugs, but without the occurrence of side effects specific to these drugs.

The existing evidence regarding the role of oxidative stress in the pathophysiology of schizophrenia, also demonstrated by us in this paper, are sufficient reasons for studying the possible therapeutic effects of antioxidants. Vitamins C and E, for example, are suitable for human clinical trials because they are convenient, inexpensive and relatively safe.

The literature on antioxidant therapy in schizophrenia divides such studies into two types: those related to psychopathology and those related to the side effects of antipsychotics. It is generally believed that specific antioxidants, such as N-acetyl cysteine, may provide obvious benefits for the clinical manifestations of schizophrenia, while, for example, vitamin E may have beneficial effects on the glycemic effects of antipsychotics (Reddy , 2010). However, fundamental clinical research studies are needed to get antioxidants to be used in the therapy of schizophrenia and its complications.

The fact that the antioxidant defense system is affected in schizophrenia, as well as the increase in lipid peroxidation observed by some studies, including the present one, offers an obvious therapeutic approach in combating the potential damage of oxidative stress by administering antioxidants.

In fact, the antioxidant defense system in mammals is an elaborate mechanism that comprises a large number of enzymatic and non-enzymatic processes. The main part is represented by the mentioned antioxidant enzymes: SOD, catalase and GPX. However, to date, no means of clinically using exogenous antioxidant enzymes have been found (Reddy, 2010). There are only a few studies that have used SOD in the form of polyethylene glycol-conjugate (PEG-SOD) in some hypoxia-reperfusion models. However, exogenous SOD cannot cross the blood-brain barrier, which obviously limits its clinical utility.

Molecules of the antioxidant defense system in humans can generally be divided into two classes, depending on their solubility: water-soluble (hydrophilic) and fat-soluble (hydrophobic). Hydrophilic antioxidants, such as vitamin C, neutralize free radicals in the cytosol, while hydrophobic antioxidants, such as vitamin E, protect cell membranes by limiting lipid peroxidation processes. In this way, non-enzymatic antioxidants are very suitable for clinical trials, being easily accessible, inexpensive and generally safe.

There has also been a particular interest in dietary antioxidants since the discovery over five decades ago that antioxidants would delay aging, prolonging life. Today there are a large number of presumed antioxidant molecules, including those derived mainly from plant sources, which are widely consumed, although their clinical efficacy is not yet fully established, some

important studies in this regard are ongoing. An example of this is Gingko biloba, which has a long history of use in traditional medicine and more recently in the clinic (Zhang et al. 2001).

However, there have been no studies that include antioxidant monotherapy for the treatment of schizophrenia. Such treatment is unlikely, in the absence of clear evidence of some primary antipsychotic effects of antioxidant molecules. Although some second-generation antipsychotics have shown some antioxidant character, it is difficult to say whether these antioxidant effects would contribute to their effectiveness. However, it is possible that the antioxidant effects mediate other neuroprotective actions, which could have implications for the effectiveness of the treatment.

Thus, there is a growing interest in the use of antioxidants as supplements or adjuvants in the primary antipsychotic treatment of schizophrenia, given the results of studies that have shown a statistical correlation between oxidative stress and low prognosis of the disease or some side effects, such as for example tardive dyskinesia.

There are numerous preliminary studies that have provided encouraging results in this regard. Thus, Dakhale and colleagues conducted a study of vitamin C supplementation in patients with schizophrenia (Dakhale et al. 2005). Forty patients were randomized to receive either placebo or 500 mg / day of vitamin C for 8 weeks. There was thus a significant reduction in the BPRS score from baseline compared to the placebo group. A significant inversely proportional correlation between plasma ascorbic acid levels and BPRS score was also described, but not between plasma MDA levels, an index of lipid peroxidation processes, and plasma ascorbic acid levels.

Berk and colleagues also examined the efficacy of N-acetyl cysteine (NAC), a precursor of glutathione, in patients with chronic schizophrenia. It should be noted that there is clear evidence of glutathione deficiency in schizophrenia, which provides a theoretical justification for such treatment. In this study, 69 subjects were randomly assigned to receive NAC and 71 subjects to be part of the placebo group. NAC was administered in divided doses, totaling 2 g / day, for 24 weeks. All subjects were further treated with antipsychotics (45% of them receiving clozapine). The result was assessed by the PANSS score (for positive and negative symptoms), and a variety of standard secondary measures, including the assessment of motor symptoms.

There was a significant reduction in positive and negative PANSS symptoms in the NAC-receiving group compared to the placebo group. Furthermore, a reduction in the severity of akathisia was observed in the group receiving NAC. Also, no difference was observed between clozapine and the other antipsychotics. Moreover, it is noteworthy that this effect was observed in a group of patients with a mean of 12 years for the duration of the disease. Berk and colleagues also succeeded in qualitatively replicating the study described above. Thus, NAC supplementation in schizophrenic patients appears to be associated with an improvement in both positive and negative symptoms.

Also, in a recent meta-analysis of adjuvant use of Ginkgo biloba extract in patients with chronic schizophrenia, a moderate effect of ameliorating negative symptoms, assessed by the PANSS and BPRS scale, was observed (Zhagh et al., 2001). Six studies (3 double-blind) were included for the pooled analyzes in this meta-analysis. A total of 466 subjects were treated with Ginkgo biloba extract and 362 subjects with placebo. The dose of Ginkgo biloba extract ranged from 120 mg / day to 360 mg / day, with a duration of treatment of at least 8 weeks. The average duration of the disease was 11 years. 90% of the study population was Chinese (where most of

the studies in this sense actually came from), and the rest were turks. These results suggest a positive effect, but larger controlled studies are needed to replicate the results.

Zhang et al. demonstrated in 2 different studies in 2001 that the addition of a Ginkgo biloba extract along with conventional haloperidol treatment resulted in an increase in antipsychotic efficacy and reduced some extrapyramidal side effects . In addition, the addition of Gingko biloba resulted in improved Scale results for negative and positive symptoms, generating a decrease in the specific activity of SOD (Zhang et al. 2001).

The use of essential polyunsaturated fatty acids has also been proposed, given the disorders of membrane phospholipid metabolism in the schizophrenic patient (Mahadik et al. 2003). Decreased levels of essential polyunsaturated fatty acids such as arachidonic acid, eicosapentanoic acid, docosapentanoic or docosahexanoic acid and their association with various psychopathological aspects have been reported in both patients receiving antipsychotic medication and patients who have never been treated. immediately after the onset of the first psychotic episode (Arvindakshan, 2003). It seems that these compounds would be of particular importance at the central level and in behavioral development. Also, given the crucial role of membrane phospholipids in the transmission of neurotransmitter signals or growth factors through receptors, we could also talk about their involvement in information processing in schizophrenic patients. Thus, some authors have reported a significant correlation between symptom severity and arachidonic and docosahexanoic acid levels in schizophrenia (Mahadik, 2003). These levels are in turn influenced by the patient's lifestyle and diet.

Also, a relatively small number of open-label studies using combinations of supplements (omega-3 fatty acids, vitamins C and E) have been performed, demonstrating some clinical benefit.

A combination of eicosapentanoic / docosahexanoic acid and vitamin C / E has been shown to result in a significant reduction in schizophrenic psychopathology, suggesting a possible use of polyunsaturated fatty acid supplements in the long-term management of schizophrenia (Arvindakshan, 2003). The use of alpha-tocopherol is also mentioned in some studies in particular for the treatment of tardive dyskinesia which may occur as a side effect of long-term treatment with antipsychotics.

Regarding the use of antioxidants to prevent or reduce the side effects of antipsychotics, there are quite a few studies, but with encouraging results.

Kim and colleagues examined the effect of alpha-lipoic acid in the treatment of metabolic syndrome in patients with schizophrenia, who had a weight gain of 10% or more from baseline. It should be noted that alpha-lipoic acid has also been used in diabetic neuropathy and several neurodegenerative disorders. In this open-label study, 7 patients (5 of whom received clozapine) were given 1200 mg / day of alpha-lipoic acid for 12 weeks. There was a significant decrease in weight of more than 3 kg, associated with a reduction of appetite. There was also a trend toward lowering fasting blood glucose and an insulin resistance index. Among serum lipids, it was observed that cholesterol levels were significantly reduced. However, there were no changes in psychiatric status. Although these findings are interesting, the open design of the study and the small sample size severely limit the interpretation of these results.

Salmasi and colleagues also examined the effect of antioxidants on the diabetic effects of olanzapine. They evaluated the effects of vitamin E on a variety of effects associated with diabetes using a double-blind, placebo-controlled study. Thirty-six patients with schizophrenia

were randomized to receive 1,200 IU / day of vitamin E or placebo. After 8 weeks, vitamin E treatment was associated with significant reductions in blood glucose.

Thus, further studies are needed to better systematize how antioxidant therapy could be applied in clinical practice. These studies, which are also part of our future concerns, should, in principle, answer some fundamental questions.

1. What antioxidants can be used?

The antioxidant defense system is an extremely complex network of antioxidant metabolites and enzymes that work together to prevent oxidative damage. There are also organs that are more sensitive to various oxidative damage. For example, the brain is most vulnerable to oxidative attacks, given its high metabolic rate and high lipid concentrations. Given these aspects, potential exogenous molecules of antioxidants should primarily have the ability to cross the blood-brain barrier.

2. What would be the effective dose?

Because most clinical trials have used fairly high doses of antioxidants, their effective administration as well as patient safety considerations are required. Thus, previous studies that used vitamin E used doses ranging from 800 IU / day to 1600 IU / day. This is in the conditions in which it was later discovered that the daily dose of vitamin E exceeding 400 IU can significantly increase the risk of mortality. Also, similar effects of beta-carotene, vitamin A and vitamin E have been demonstrated, but not of vitamin C and selenium.

3. Should the administration be monotherapeutic or in combination?

In this regard, most previous studies have used, for example, vitamin E as monotherapy. However, it is well known that vitamin C is used to regenerate vitamin E from the peroxyl stage. In the absence of vitamin C, vitamin E can even act as a potential antioxidant. Thus, the whole prooxidant-antioxidant assembly of the organism and the numerous chemical interactions necessary for the activity of a molecule to be antioxidant and efficient must be taken into account.

4. What should be the minimum duration of treatment?

Related to this, Berk and colleagues found that N-acetyl cysteine had no effect on negative symptoms after 8 weeks of treatment. Continuing the study for up to 24 weeks, they then concluded that the maximum improvement in the patient's condition was observed in the 22nd week. Thus, whenever possible, the studies should be performed for as long as possible. within general safety limits, to determine the duration of response to treatment and post-treatment and to better understand the durability of the effects of this treatment. Also, in this way the various theoretical bases of the potential antioxidant therapy will be better developed.

5. When should treatment be started?

Obviously, combating the side effects that occur as a result of the administration of antipsychotics requires the initiation of antioxidant supplementation from the beginning of treatment, as evidenced by the results of the study led by Dorfman Etrog and colleagues (19). It is also recommended that antioxidant supplementation of treatment be initiated as early as possible after the onset of the disease (Reddy, 2010).

6. Should antioxidant treatment be combined with omega-3 fatty acid supplements?

There is also recent evidence of certain omega-3 fatty acid deficiencies in schizophrenia, and moreover, studies that state that supplementation with omega-3 fatty acid treatment improves the overall clinical condition in schizophrenia. These acids also appear to possess

certain antioxidant properties. This was demonstrated in particular by Berger et al., who showed that ethyl-eicosapentaenoic acid, administered for 12 weeks to patients with the first psychotic episode, was associated with increased glutathione levels in the brain. They also showed an inversely proportional correlation between glutathione levels and negative symptoms. Given that oxidative stress leads to deficiencies of omega-3 fatty acids in the membrane, supplementation with this type of acid antioxidant therapy could play an important role in improving the clinical picture of schizophrenia. These aspects are also supported by studies that found a low level of postmortem GSH in the prefrontal cortex of patients with schizophrenia.

In fact, there are already two studies in this regard, in which a combination of omega-3 fatty acids and vitamins E and C were administered, which was tolerated without problems by patients with schizophrenia and, moreover, resulted in a reduction of general psychopathological symptoms.

However, more rigorous studies are needed to evaluate the usefulness of this association, given the aforementioned issues regarding the complexity of biochemical systems and the numerous interactions between antioxidant and prooxidant molecules.

Thus, considering that our clinic also conducted studies on the effect of various agents with antioxidant effect (L-lysine, omega-3 fatty acids in fish oil, administration of Bilobil, etc.), as well as the results in the present study, there are all the prerequisites for a complex study on the effects of the administration of antioxidants in patients with schizophrenia, to answer as many of the fundamental questions in this field, mentioned above.

In addition, considering the latest developments on COVID-19 pandemics, I am also planning some important future papers/studies on the neuropsychiatric manifestations associated with COVID-19 infection, and its effects on the oxidative stress status, since the new coronavirus was recently correlated for example with NOX 2 activity (Sindona et al, 2021) or other oxidative stress metabolism related mechanisms (Cechini et al., 2020, Forcados et al., 2021).

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